The clinical features, microbiology, and genomics of neonatal sepsis in a children's hospital in Vietnam

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Declaration

The work presented in this thesis is my own and was conducted under the supervision of my supervisor Professor Stephen Baker at the Oxford University Clinical Research Unit in Vietnam. Information from this study has not been submitted for any degree or other qualification elsewhere.

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The clinical features, microbiology, and genomics of neonatal sepsis in a children's hospital in Vietnam

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Abstract

Sepsis is a common and deadly condition affecting the neonatal population. I aimed to understand the causes and outcomes of neonatal sepsis at Children's Hospital 1 in Ho Chi Minh City, Vietnam. A retrospective study from 2013 to 2016 found that *Acinetobacter* spp., *Escherichia* coli, *Klebsiella* spp., *Pseudomonas* spp., coagulase-negative staphylococci (CoNS), *Staphylococcus aureus*, and *Streptococcus pneumoniae* were the most common organisms. These organisms were resistant to a wide variety of antimicrobials, which may impact on the efficacy of antimicrobial therapy. I next conducted a prospective study of sepsis in 524 neonates with 69 deaths from January 2017 to June 2018. This group had a mortality of 13.2%; sclerema, leukopenia <4,000/mm^3, thrombocytopenia <100,000/mm^3, base excess < –20 mEq/L, lactate >4 mmol/L, and hyperglycaemia >180 mg/dL, were associated with death. The major organisms (from 405 isolates) included *Klebsiella* spp. (6.9%), *Escherichia coli* (6.7%), *Acinetobacter* spp. (4.0%), *Enterobacter* spp. (3.5%), CoNS (57.3%), *Staphylococcus aureus* (4.4%), and *Streptococcus* spp. (2.5%). These organisms were highly resistant to all non-carbapenem antimicrobials. The genomics of 15 *Acinetobacter baumannii* identified sequence type ST570 within genomic complex 2 in 80% of isolates and found the $bla_{\text{OXA-23}}$, bla_{MBL}, bla_{ADC}, bla_{A2}, *mphE*, *msrE*, and *armA* AMR genes. A common transposon, carrying $bla_{\text{OXA-23}}$ was associated with widespread carbapenem resistance. In this location, neonatal sepsis was associated with high mortality, complicated clinical features and caused by caused by differing MDR bacteria.

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Abbreviations

3GC	Third-generation cephalosporin
95% CI	95% Confidence Interval
95% HPD	95% Highest Posterior Density
AAP	American Academy of Paediatrics
AMC	Amoxicillin-clavulanic acid
AMK	Amikacin
AMP	Ampicillin
AMR	Antimicrobial resistance
BSC	Balanced scorecard
BSIs	Bloodstream infections
CAS	Community-acquired sepsis
CAZ	Ceftazidime
CH1	Children's Hospital 1
CIP	Ciprofloxacin
CL	Chloramphenicol
CLSI	Clinical and Laboratory Standards Institute
CM	Clindamycin
CoNS	Coagulase-negative staphylococci
CRE	Carbapenem-resistant Enterobacteriaceae
CRO	Ceftriaxone
CRP	C-reactive protein
CT	Colistin
CTX	Cefotaxime
DeNIS collaboration	Delhi Neonatal Infection Study collaboration
E	Erythromycin
EMA	European Medicines Agency
EOS	Early-onset sepsis
ESBL	Extended spectrum beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FEP	Cefepime
FOX	Cefoxitin
GBS	Group B *Streptococcus*
GC	Genomic Complex
GEN	Gentamicin
GLASS	Global Antimicrobial Resistance Surveillance System
HAS	Hospital-acquired sepsis
HCMC	Ho Chi Minh City
HTD	Hospital for Tropical Diseases
I/T ratio	Immature neutrophil count/total neutrophil count ratio
ICH GCP	International Council on Harmonization Guidelines for Good Clinical Practice
ICUs	Intensive care units
IL	Interleukin
IMP	Imipenem
IQR	Interquartile range
IRB	Institutional review board

LEV	Levofloxacin
LMICs	Low- middle income countries
LOS	Late-onset sepsis
LZD	Linezolid
MDR	Multi-drug resistance
MEM	Meropenem
MIC	Minimum inhibitory concentrations
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MSPE	Mean-squared prediction error
NA	Not available
NA	Nalidixic acid
NA	Not available
NeoAMR research network	Neonatal antimicrobial resistance research network
NeonIN surveillance network	Neonatal infection surveillance network
NICHD	National Institute of Child Health and Human Development
NICUs	Neonatal intensive care units
NTISS	Neonatal Therapeutic Intervention Scoring System
OLS	Ordinary least squares
OR	Odds ratio
OUCRU	Oxford University Clinical Research Unit
OX	Oxacillin
OxTREC	Oxford Tropical Research Ethics Committee
P	Penicillin
PCR	Polymerase chain reaction
PCT	Procalcitonin
PEF	Pefloxacin
PTX	Pentoxifylline
RD	Rifampicin
ROC Curve	Receiver Operating Characteristic Curve
SEA	Southeast Asia
SNP	Single nucleotide polymorphism
spp.	Species
ST	Sequence type
STROBE-NI	Strengthening the Reporting of Observational Studies in Epidemiology for New-born Infection
SXT	Trimethoprim-sulfamethoxazole
TCC	Ticarcillin-clavulanic acid
TE	Tetracycline
TNF	Tumour necrotic factor
VAN	Vancomycin
VAP	Ventilator-associated pneumonia
WHO	World Health Organisation
XDR	Extensive drug resistance

1 Introduction

1.1 Background

The neonatal period extends from birth to day 28 of life and represents an important transition from the uterine to the outside environment. In this first stage of life, infection is a leading cause of mortality and morbidity (1–3). Neonatal infections are infections that affect a child in the neonatal period with pathogens infected before, during, or after birth. The burden of neonatal infections varies by geographic region and it is globally estimated that annually more than 1.4 million neonatal deaths are the consequence of infection (4,5). Globally, a recent population-level estimate for sepsis in neonates was 2,202 (95% CI 1,099–4,360) per 100,000 livebirths, with mortality ranging from 11% to 19% (6). Generalising these numbers on a global scale, suggests an estimated 3 million cases of neonatal sepsis per year (6). These figures show that sepsis is a common and deadly condition affecting the neonatal population globally. In low- middle income countries (LMICs), neonatal sepsis is one of the leading causes of neonatal admission, morbidity, and mortality (7,8). Additionally, there are increasing number of multi-drug resistant (MDR) bacteria associated with sepsis (7,8), that may take us back to the pre-antibiotic era; therefore, the management of neonates with sepsis in developing countries is becoming increasingly challenging (7,8).

Neonatal sepsis is widely accepted as a clinical syndrome of systemic inflammation in response to, or occurring the same time as, a possible or proven infection (frequently by identifying bacterial bloodstream infection) occurring in children ≤28 days of age (1–3). However, a precise consensus definition and diagnosis of neonatal sepsis remains a challenge (9,10). Early-onset sepsis (EOS) is commonly defined as the start of sepsis

manifestation within 72 hours of birth (11–14), and is often associated with vertical transmission of pathogens during delivery as a result of chorioamnionitis or maternal genital tract colonization (11). Late-onset sepsis (LOS), which occurs after 72 hours of birth (15–18), may also be caused by similar vertical transmission, or horizontal transmission due to direct contact with the surrounding environment, attendant healthcare staff, or invasive procedures (15).

Southeast Asia (SEA) is comprised of 11 countries: Brunei, Cambodia, East Timor, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, and Vietnam. These countries vary greatly in their political, social, economic and healthcare demographics. In SEA, it has been estimated that neonatal sepsis accounts for approximately 16% of neonatal mortality with data varying among different countries in the region (19). SEA accounts for almost 30% of global mortality in neonates, and many countries in this region have not met the Millennium Development Goal 4, which aims to reduce the under-five mortality rate by two-thirds between 1990 and 2015 (20). Over the past twenty years, many countries is this region have developed programs to improve the health of new-borns and one of the most important aim is to reduce deaths due to neonatal bloodstream infections (BSIs) (20).

1.2 The epidemiology of neonatal sepsis

Although the incidence of neonatal sepsis is reported as 1 to 5 cases in 1,000 live births in developed countries, other population-based studies in the developing countries suggest the incidence of neonatal sepsis ranged from 49 to 170 cases per 1,000 live births (21). The risk of neonatal sepsis increases with the decrease of gestational age and birth weight (22,23). The NeonIN (Neonatal Infection) surveillance network of 12 English neonatal

units with neonates admitted from 2006 to 2008 reported that the majority of infections occurred in premature (82%) and low birth weight (81%) neonates (22). In this English study, the incidence of EOS was 0.9/1,000 live births and 9/1,000 neonatal admissions while the incidence of LOS was 3/1,000 live births and 29/1,000 neonatal admissions (22). The National Institute of Child Health and Human Development (NICHD) neonatal research network estimated that the incidence of EOS in very low birth weight neonates has remained stable while the incidence of LOS decreased for preterm infants at 26 neonatal centres in the United States between 1993 and 2012 (23).

In previous studies of neonatal sepsis originating from LMICs in SEA, the condition was found to be the most prevalent cause of morbidity (24,25). Sepsis was the secondly most common (35%) neonatal morbidity from data of a study in KK Women's and Children's Hospital in Singapore between 1990–2007 (24). Furthermore, sepsis accounted for the most diagnosis at admission (38%) according to a retrospective study of 2,088 hospitalised neonates at National Hospital Guido Valadares, Dili in East Timor (25).

Sepsis is also a major cause of death in neonates in SEA (25–29). The mortality of neonatal sepsis was 26% from data of an 3-year observational study in East Timor (25). Furthermore, a study performed in Cambodia from 2007 to 2011 reported an overall mortality of 36.9% for neonatal BSIs (29). In Vietnam, neonatal sepsis has been similarly observed as a common condition with an associated high mortality rate (26–28). A 12-month prospective study performed in a children’s hospital in the south of Vietnam identified a 16.1% (62/385) mortality rate in neonates with BSIs (26). A further prospective cohort study, conducted in the largest neonatal unit in central Vietnam, reported the isolation of a bacterial pathogen from a blood culture in 115 (16%) cases

among 616 neonatal patients with 729 episodes of suspected sepsis. The overall case fatality rate for microbiologically confirmed sepsis in this study was 46% (27). Figure 1.1 shows the location of some hot spots of new-born sepsis in SEA in term of high mortality.

Figure 1.1 Locations with high mortality of neonatal sepsis in SEA

This figure shows the location, time of study, and mortality rate of neonatal sepsis in Southeast Asia. The red dots and their size show the mortality percentages of neonatal sepsis in different places in SEA (25–29). The size of the red dots is directly proportional to the percentage of mortality; a mortality rate of 46% was observed in Da Nang, Vietnam in 2015 (27).

In consideration of the onset of sepsis, LOS has been generally found to be more frequent than EOS in SEA (30–32). Al-Taiar and colleagues revealed that the incidence of EOS was 0.62 per 1,000 live births, or 4.91 per 1,000 admissions; while the incidence of LOS was 5.00 per 1,000 live births, or 21.22 per 1,000 admissions (30). Among 3,880 infants admitted to neonatal intensive care units (NICUs) in Malaysia, 623 cases (16.1%) were diagnosed with sepsis in which 61 cases were EOS (9.8%) and 562 cases were LOS (90.2%) (31). The incidence rates of EOS caused by group B *Streptococcus* (GBS) were 0.2 and 0.3 per 1,000 live births in Thailand and the Philippines, respectively (32). Neonatal sepsis due to GBS is not frequently identified in SEA, which is distinct to western countries. Over a six-month period, there were 2 cases (1 died) of EOS due to GBS among 8,409 live births in Thailand and 3 cases (2 died) of EOS among 11,768 live births in the Philippines (32).

It is estimated that children born in in healthcare settings in LMICs are at 3–20 fold higher risk of severe neonatal infections than those in higher-income countries (33). This observation shows that sepsis is not an abstract problem and is a significant cause of morbidity and mortality in the neonatal population, as has been highlighted by many studies in SEA. Table 1.1 summarizes the main findings in term of morbidity, mortality and incidence rate of neonatal sepsis in SEA.

Table 1.1 Morbidity, mortality and incidence of neonatal sepsis in Southeast Asia

Authors	Location	Time	Study	Main findings
Agarwal P et al. (24)	KK Women's and Children's Hospital, Singapore	1990–2007	A retrospective cross-sectional study	Sepsis was the secondly most common morbidity (35%)
Bucens IK et al. (25)	National Hospital Guido Valadares, East Timor	2008–2010	A retrospective study of 2088 hospitalised neonates	Most common morbidity was sepsis (38%)
Stoesser N et al. (29)	Siem Reap, Cambodia	2007–2011	A retrospective study	Mortality rate of neonatal sepsis was 36.9%
Turner C et al. (34)	Maela Camp, Thailand-Myanmar border	2009–2012	An observational study	Incidence of EOS was 0.7 per 1,000 live births (95% CI 0.1–2.1)
Kruse AY et al. (26)	A children's hospital in Vietnam	Published in 2013	A 1-year prospective study	Among 385 neonates. 62 died due to sepsis (mortality rate 16.1%)
Al-Taiar et al. (30)	Neonatal care units in Malaysia, Thailand	Published in 2013	A prospective cohort study of 963 episodes of neonatal sepsis	Incidence of EOS was 0.62 (95% CI 0.45–0.82) per 1,000 live and of LOS was 5.00 (95% CI 4.51–5.53) per 1,000 live births. Mortality was 7.0% (95% CI 3.9%–12.0%) for EOS and 16.0% (95% CI 13.7%–19.0%) for LOS
Tran HT et al. (27)	A neonatal unit in Vietnam	Published in 2015	A 1-year prospective study	The mortality rate of sepsis was 46%
Villanueva-Uy ME et al. (32)	Hospitals in the Philippines and Thailand	Published in 2015	A 6-month prospective of neonatal sepsis due to GBS	Incidence rates of GBS EOS were 0.2 (95% CI 0.0–0.8) and 0.3 (95% CI 0.1–0.8) per 1,000 live births in Thailand and the Philippines, respectively. No cases of LOS
Boo NY et al. (31)	34 Malaysian NICUs	2016	A retrospective study	61 EOS (9.8%) and 562 LOS (90.2%). Overall median EOS rate was 1.0% (IQR 0%, 2.0%)

1.3 The progression to neonatal sepsis

EOS is often caused by vertical transmission of pathogens as a result of chorioamnionitis or infections of bacteria colonizing at the gastrointestinal or genital-urinary tract of mother during delivery (35). In 1959, Benirschke demonstrated that infection caused by bacteria residing in the maternal vagina is the most common route of early-onset neonatal bacterial infection (36). Maternal chorioamnionitis is also a well-recognized risk factor for early-onset neonatal sepsis as the pathogenesis of EOS has long been recognized as its initiation from the amniotic cavity to the foetus (35). The usage of forceps during delivery and electrodes placed for intrauterine monitoring have been associated with EOS because they penetrate the neonatal defensive epithelial barriers (37).

LOS can be caused by two mechanisms. Vertical transmission from mother to neonate results in colonisation of the neonatal; bacteria colonising the intestinal and nasopharyngeal tract of the neonate then trigger sepsis (38,39). Horizontal transmission, due to direct contact with the surrounding environment or healthcare providers, in which any invasive procedures or interventions (surgery or intravascular catheterization) increase the risk of LOS (40). Disruption of the intact skin or mucosa, as the results of invasive procedures (e.g. intravascular catheter), also increases the risk of LOS (41).

Factors such as respiratory failure, unstable body temperature, hyperglycaemia, hypoglycaemia, and abnormal metabolic balance have been demonstrated to increase the severity of neonatal sepsis (42,43). Metabolic factors, including hypoxia, acidosis, hypothermia, and inherited metabolic disorders (e.g., galactosaemia), also contribute to the risk of neonatal sepsis. These factors are thought to disrupt the host defences (i.e., such as the innate immune response) (37,43).

1.4 Risk factors for neonatal sepsis

Most EOS is caused by ascending colonization and subsequent infection of uterine compartment including amniotic fluid, placenta, umbilical cord and foetus with organisms from the genital-urinary or gastrointestinal tracts of the mother (44). Therefore, the risk factors for EOS are commonly related to the presentation of the maternal genital-urinary tract infections, perinatal distress, amniotic fluid and placental infection (44–46). LOS is caused by the horizontal transmission as the result of direct exposure with healthcare providers or infectious sources in the hospital environment (47,48). In addition, the disruption of the intact skin or mucosa, invasive procedures or surgeries, and prolonged use of ventilators, fluid infusion, and specific drugs have been found to be risk factors of LOS (47–49). Neonates born preterm and have low birth weight have higher risk of sepsis (50,51). The risk factors for neonatal sepsis globally are summarized in Table 1.2.

Table 1.2 Risk factors of neonatal sepsis globally

	Risk factors (44–51)
Early-onset neonatal sepsis	Maternal urinary tract infection within 1 month before delivery Maternal fever due to infections within 2 weeks before delivery Maternal fever during delivery or 24-hour ante- or post-partum Maternal group B streptococcal infection Evidence of foetal distress Apgar score at 5-minute ≤6 Multiple birth Chorioamnionitis Abscess lesions of placenta (*Listeria monocytogenes*) Early rupture of membranes >12 hours Prolonged rupture of membranes >18 hours
Late-onset neonatal sepsis	Disruption of skin or mucosal barrier Prolonged use of intravascular catheterization Invasive procedures or surgery Necrotizing enterocolitis Prolonged use of antimicrobials Prolonged use of H_2 receptor antagonist or proton pump inhibitor Prolonged use of mechanical ventilation Prolonged use of total parenteral nutrition Prolonged length of stay
Neonates	Premature (<37 weeks of gestational age) Low birth weight (<2,500 g)
Environment	Bad aseptic conditions at hospital-based healthcare Invasive procedures Inadequately aseptic procedures Overload of hospitalization Bad hygienic conditions at home-based healthcare

Although data for risk factors of neonatal sepsis in SEA are limited, studies in this region have identified factors that increase the risk of sepsis in neonatal population. These factors are related to home delivery and low standards of delivery care, maternal factors, prematurity, no breast milk feeding, sleeping with hot coals under beds, central vascular line, ventilator equipment, antimicrobial bacterial colonisation, sputum suctioning, history of using antibiotics, steroids, and surfactant (31,52–60). The risk factors of neonatal sepsis locally in SEA are summarized in Table 1.3.

Table 1.3 Risk factors of neonatal sepsis locally in SEA

Authors	Location	Time	Study	Risk factors (31,52–60)
Lee JKF et al. (52)	Kuala Terengganu Hospital, Malaysia	2001–2002	2 outbreaks of *Burkholderia cepacia*	A prior long line (OR=7.07, 95% CI 1.37–36.47, *p*=0.019).
Quiambao BP et al. (53)	In Bohol Island, Philippines	2007	A study of risks factors of Gram-negative neonatal bacteria sepsis	Neonates <7 days of age (*p*=0.002) and home delivery (*p*=0.012)
Awaisu A et al. (54)	Hospital Universiti Sains Malaysia	Published in 2007	A study described the clinical features and outcomes	Maternal factors were the most common (37.2%)
Tan JH et al. (55)	KK Women's and Children's Hospital in Singapore	2005–2008	A retrospective study comparing morbidity and mortality between neonatal groups	Late preterm increased risks of sepsis (1.7% vs. 0.6%) compared to term neonates
Litzow JM et al. (56)	2 largest NICUs in Manila, Philippines	Published in 2009	A 10-month prospective study of colonization and bloodstream infection by Gram-negative bacteria	Colonization with a drug-resistant Gram-negative pathogen (OR=1.4, 95% CI 1.0–1.9)
Hengstermann S et al. (57)	In the Philippines	Published in 2010	A case control study	No breast milk (OR=4.9, 95% CI 1.3–18.3)
Anderson M et al. (58)	Mahosot Hospital in Vientiane, Laos	2000–2011	Data of sepsis with traditional putting hot coals under beds	Sleeping on hot bed associated with *S. aureus* sepsis (OR=4.8; 95% CI 1.2–19.0)
Sobel HL et al. (59)	A Philippines hospital	Published in 2011	An observational assessment of causes of nosocomial neonatal sepsis	Drying, kangaroo mother care, delayed cord clamping, breastfeeding and delayed bathing at lower level than WHO standards

Table 1.3 Risk factors of neonatal sepsis locally in SEA (continued)

Authors	Location	Time	Study	Risk factors (31,52–60)
Boo NY et al. (60)	NICU of Universiti Kebangsaan Malaysia Medical Centre, Malaysia	Published in 2015	A case-control study to determine factors associate with CoNS sepsis in neonates.	Central venous lines (OR=5.8, 95% CI 1.9–17.8, p=0.002); >2 nasopharyngeal suctioning 48h before positive culture (OR=7.3, 95% CI 3.3–16.2, p<0.001). Suctioning was only factor associated with CoNS sepsis (OR=20.8, 95% CI 3.5–125.3, p=0.001)
Boo NY et al. (31)	34 Malaysian NICUs in the Malaysian National Neonatal Registry	Published in 2016	A retrospective study of 3,880 very low birth weight new-borns to determine factors associated with inter-institutional variations in sepsis rates of neonates	Compared with NICUs with no EOS (n=14), NICUs with EOS (n=20) had higher rates of patient loads; history of antenatal steroids, intrapartum antimicrobials, surfactant use, pneumonia and central venous catheterization.

1.5 The clinical manifestation of neonatal sepsis

The clinical features of neonatal sepsis are various, subtle, and nonspecific. Signs and symptoms of sepsis in neonates include: “unhealthy” or neonates with ill-appearance, temperature instability (fever or hypothermia), inadequate weight-gain or weight-loss, respiratory distress (tachypnoea, grunting, flaring of the nasal alae, retractions, and decreased breath sounds), cardiovascular disturbances (tachycardia, hypotension, prolong capillary refilled time, cyanosis, mottled skin, cold limbs), neurological abnormalities (lethargy, decrease of movement, irritation, hypertonia or hypotonia, seizures, and bulging fontanelle), gastrointestinal abnormalities (anorexia, diarrhoea, vomiting, abdominal distention, and feeding intolerance), haematological manifestations (petechiae, purpura, bleeding, hepatomegaly, and splenomegaly), skin and mucous membranes (purulent or malodorous discharge of the ears or skin, bullous impetigo, cellulitis, omphalitis, rash, jaundice, pale) (9,12,45,46,49,61,62).

In SEA, various signs and symptoms or associated conditions have been suggested as clinical indications of neonatal sepsis, these include exanthematous maculopapular rash (63), meningitis, osteomyelitis (64), urinary tract infection, prolonged neonatal jaundice (65), and high mean heart rate (66). Viswanathan and co-workers observed an outbreak of sepsis in 10 neonates within a 2-week period in 2011 in a NICU in Thailand (63). In this study new-born babies presented with exanthematous maculopapular rash associated with Gram-positive bacteria (63). An 18-day-old new-born baby with an infected cephalohematoma caused by an extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and complicated with septicaemia, meningitis, and skull osteomyelitis was also reported in Thailand in 2011 (64). In another study from Malaysia, an unusual presentation of urinary tract infection and prolonged jaundice in a case of LOS due to

group B beta-haemolytic *Streptococcus* was reported (65). Researchers in a study from Vietnam observed variable heart rate during automatically selected stationary periods and recognised that the high mean heart rate was associated with neonatal EOS in a prospective operational study (66).

The clinical features of neonatal sepsis are commonly nonspecific, which poses a significant challenge for clinicians to reliably diagnose this condition. Additionally, non-septic manifestations of other conditions may mimic the clinical features of sepsis in neonates, and septic and non-septic aetiologies may co-occur in the same patient (62,67). For all of these described reasons, the diagnosis of neonatal sepsis is a multifactorial process, in which diagnostic investigations should be performed to provide additional evidence for neonates presenting with sepsis-related risk factors, signs, and symptoms (68).

1.6 The laboratory investigations for neonatal sepsis

In our practice, it is recommended that other investigations should be performed to provide more information in any neonates having sepsis-attributable risk factors, signs or symptoms. At Children's Hospital 1 (CH1) in Ho Chi Minh City (HCMC), a "sepsis panel" including a complete blood count, C-reactive protein (CRP) value, and blood culture is used routinely for the diagnosis and treatment of sepsis in neonates.

1.6.1 Non-culture-based diagnostics

A complete blood count may also be helpful for sepsis diagnosis when it is obtained > 6 hours after delivery (68–70). In CH1, haematological characteristics that are thought to be related to neonatal sepsis include total white blood cells <6,000/mm^3 or >30,000/mm^3 (<24 hours of age), 5,000/mm^3 or >20,000/mm^3 (≥24 hours of age), neutrophil count <1,000–1,500/mm^3 indicating a poor outcome, band neutrophil count >10%, immature neutrophil count/total neutrophil count (I/T) ratio >0.2, and the presence of Dohle bodies, toxic granules or vacuoles (68–70). A platelet count <150,000/mm^3 is considered to be associated with sepsis in neonates, and if this value <100,000/mm^3 the outcome of sepsis is likely to be worse (68–70). When interpreting haematological values, other associated problems particularly haematological diseases and the use of immunosuppressive drugs (e.g. corticosteroids) should also be considered (68).

C-reactive protein (CRP) is produced in the liver and is a common but nonspecific biomarker of inflammation and tissue injury (68,71). A value of CRP >10 mg/L may indicate an infection and has a sensitivity of ~90% for neonatal sepsis (68,71). However, CRP is not specific for sepsis because other conditions, such as foetal distress, injury, meconium aspiration, and intracranial haemorrhage can also lead to an increase of CRP value (68,71). CRP hs (high sensitivity) can be detected at lower value (level 0,01 mg/L) (68,71). CRP increases 4–6 hours after inflammatory initiation, and doubles in blood concentration every 8 hours, reaching the highest value after 36–48 hours (68,71). The half-life of CRP is 19 hours and may continue to increase within 24–48 hours after the onset of infections even after antimicrobials have been administered (68,71). The value of CRP is elevated in neonatal sepsis and meningitis. In addition to the CRP value, clinical scenario, and the results of other laboratory findings must be considered to support a

diagnosis of sepsis. Serial values of CRP at 6 hours of age, 12 hours of age and every 2–5 days have been used at our institution (CH1) for the follow-up of sepsis progression and treatment response. A CRP value may also be useful for clinicians to determine the duration of antimicrobial therapy when the result of blood culture is not available or is negative in an unstable patient with sepsis (68,71). Data from Thailand found that serial CRP may be suitable for guiding antimicrobial therapy and limiting the antimicrobial overuse in neonatal sepsis (72). In this study, a primary CRP value >19 mg/L and a second CRP value >12.5 mg/L had the sensitivity of 92.6% and 96.3%, respectively, for the prediction of neonatal sepsis (72).

Procalcitonin (PCT) is released by C cell in the thyroid gland (68,73,74). PCT is elevated in neonatal sepsis, meningitis, pneumonia, and urinary tract infections (73,74). A concentration of PCT from 2 to 10 ng/mL is an indication of infection and a PCT value >10 ng/mL is a strong predictor of neonatal sepsis (73,74). The pooled sensitivity and specificity of procalcitonin for early detection of neonatal sepsis in a meta-analysis of 16 studies was 81% and 79%, respectively (74). PCT increases within 4 hours after the onset of bacterial infection, reaches a plateau within 6–8 hours and continues to increase for at least 24 hours after onset of infection (73,74). The half-life of PCT is approximately 25–30 hours (73,74). Many observational studies have suggested that PCT may be an equal to or better than CRP as a biomarker for the detection of bacterial infections in neonates (73,74). Although procalcitonin has its own value for the detection of bacterial infections in neonates, there is not enough evidence for this marker to be a diagnostic or routine investigation for neonatal sepsis (73–75). In Malaysia, a PCT cut-off level of at least 2 ng/mL has been suggested for the diagnosis of neonatal sepsis; the sensitivity and specificity of PCT in detecting neonatal sepsis was 88.9% and 65.2%, respectively (76).

Presepsin, a CD14 subtype, is a further promising marker that increases in the response to bacterial infection (77). In a study by Iskandar, it was concluded that presepsin may perform better than PCT for the early diagnosis of neonatal sepsis (77).

Pro-inflammatory cytokines such as interleukin 2 (IL-2), IL-6, gamma interferon (gamma-INF), alpha tumour necrotic factor (alpha-TNF) and anti-inflammatory cytokines (IL-4 and IL-10) are all known to increase during bacterial infection (68). However, the cost of these cytokine assays is high, and no cytokine is reliable as a sole or main marker of sepsis (68). A prospective study performed at the NICU of Harapan-Kita Women and Children's Hospital, Jakarta, Indonesia suggested that IL-6, IL-8, IL-15, and alpha-TNF may be the more useful predictors of LOS (78).

There is lack of a consensus of indications for cerebral spinal fluid analysis and culture in neonatal population (42,68). In 2012, American Academy of Paediatrics (AAP) recommended that lumbar puncture should be performed for a neonate having any of the conditions including a positive blood culture, clinical manifestation of sepsis or meningitis, laboratory results leading to suspicion of sepsis or meningitis, or when the patient condition becomes worse even under antibiotic therapy and no source of infection is identified (42,68).

The investigation of nasotracheal aspirations, urine, stool or other biological samples for detecting possible sources of sepsis and associated infections may also be considered in accordance to individual conditions of patients (68). In CH1, other investigations including blood gases, liver enzymes, serum creatinine, X-ray, ultrasound, etc. are often conducted to evaluate the organ dysfunctions in sepsis and underlying diseases (79–82).

1.6.2 Culture based diagnostic of neonatal sepsis

A positive blood culture in a patient having clinical signs and symptoms of sepsis is the gold standard for the diagnosis of sepsis. The sensitivity of blood culture depends on the number of blood cultures performed and the volume of blood used (68,83,84). In practice, waiting for the results of multiple blood cultures may delay the use of appropriate empirical antimicrobials. Therefore, a single blood culture is requested before the empirical antimicrobial therapy is initiated (68,83,84). The sensitivity of blood culture may be impaired because of an inability to obtain adequate a blood sample volume for culture, especially from sick very low birth weight infants. In addition, the use of antepartum antimicrobials may negatively impact blood culture sensitivity. A minimum volume of 0.5 mL of blood is required for blood cultures, but a higher volume may be required to detect organisms in low concentration (<4 colony forming units/mL) (68,83,84).

1.7 Bacterial aetiology of neonatal sepsis

Globally, GBS and *Escherichia coli* are the two most common causes of EOS in neonates (22,46). In a large study of EOS conducted by the National Institute of Child Health and Human Development (NICHD), the incidences of EOS caused by *Escherichia coli* and GBS in neonates with very low birth weight were 5.1 and 2.1 per 1,000 live births, respectively. For neonates with birth weights ranging from 1,500 to 2,500 g, the incidences were 0.5 and 0.4 per 1,000 live births, respectively (46).

The most common pathogen of LOS is CoNS particularly in premature new-borns. In the study of LOS performed by NICHD, nearly half of the LOS in low birth weight neonates were caused by CoNS (48%), followed by 22% of other Gram-positive bacteria including *Staphylococcus aureus*, Enterococci, GBS; and 18% of Gram-negative bacteria including *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Serratia*, and fungi (12%) (17). Particularly, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas aeruginosa* are significantly associated with LOS in neonates admitted to NICUs (18). Globally, many studies have investigated the bacterial profile of EOS and LOS in neonates (9,12,17,18,22,45,46,49,61) and the most common pathogens are shown in Table 1.4.

Table 1.4 Common pathogens of early- and late-onset sepsis in neonates globally

Early-onset neonatal sepsis (9,12,17,18,22,45,46,49,61)	**Late-onset neonatal sepsis** (9,12,17,18,22,45,46,49,61)
Gram-positive bacteria	**Gram-positive bacteria**
GBS	CoNS
Listeria monocytogenes	*Staphylococcus aureus*
Enterococcus	*Enterococcus*
Other streptococci: *Streptococcus pyogenes*, *Streptococcus viridans*, *Streptococcus pneumoniae*	
Gram-negative bacteria	**Gram-negative bacteria**
Escherichia coli and other enteric Gram-negative bacteria	*Escherichia coli*
	Klebsiella spp.
Non-typed *Haemophilus influenzae*	*Pseudomonas aeruginosa*
	Enterobacter spp.
	Citrobacter spp.
	Serratia spp.
	Candida spp.

In SEA, the diagnosis of neonatal sepsis is commonly confirmed by positive blood cultures in a neonate having signs or symptoms of sepsis (27,58,85,86). In Vietnam, a study in Da Nang found that a pathogen was isolated in 115 (16%) episodes in 106 neonates, CoNS and *Staphylococcus aureus* were most common in EOS, while *Acinetobacter*, CoNS, and *Klebsiella pneumoniae* were most common in LOS (27). Another study of all positive blood cultures among neonates admitted to a tertiary paediatric hospital during a 12-month period in Vietnam found the most common Gram-negative pathogens were *Klebsiella* spp. (n=78, 19.5%), *Acinetobacter* spp. (n=58, 14.5%) and *Escherichia coli* (n=21, 5.2%). Only 3 *Streptococcus* spp. (0.8%) were isolated but GBS was not isolated (26).

In term of CoNS, we need to evaluate the clinical relevance (prematurity, low birth weight, indwelling intravascular catheters, invasive procedures or interventions, skin infection, response to antimicrobials) and the independent assessment of two microbiologists to confirm the diagnosis of sepsis due to this pathogen. An appropriately powered study of CoNS is required to determine to what extent they are colonising organisms or pathogens, the identification of these organisms remains a major challenge of the diagnosis of neonatal sepsis. A case-control study regarding CoNS colonisation and/or sepsis in neonates was performed in neonates admitted to the NICU of Universiti Kebangsaan Malaysia Medical Centre, Malaysia (60). Pure growth of CoNS cultured from the peripheral blood sample of relevant symptomatic infants was the criteria for the diagnosis of CoNS sepsis. In this study, CoNS colonisation was detected in 113 (8.7%) cases. CoNS sepsis was found in 12 cases with CoNS colonisation (10.6%) and 7 neonates without CoNS colonisation (0.6%) (60).

In SEA, limited studies of GBS serotypes and evaluation of antibodies against GBS have been conducted. In Malaysia, GBS were isolated during a study of 200 pregnant women (87). Serotypes V (19%) and VI (17%) were the most frequent, followed by serotypes III (12%), Ia (11.5%) and IV (10%); 17% of the isolates were non-typeable (87). The recognition of new serotypes of GBS serotypes has significance for the development of vaccine to prevent infections caused by GBS (87). A research study in which 520 paired mother and cord serum samples were obtained at the time of birth was conducted on the Thailand-Myanmar border (88). Neonates born to mothers carrying serotype II at birth showed higher antibody-mediated C3b/iC3b deposition against serotype II than neonates born to mothers with no serotype II carriage (88). An assessment of antibody-mediated C3b/iC3b deposition against GBS may inform the sero-epidemiology of anti-GBS antibodies in mothers and neonates. This study generated the possibility of measuring antibody-mediated complement deposition against different GBS serotypes in larger studies (88). A cross sectional study of 549 pregnant women over a 13-month period on the Thai-Myanmar border found that GBS carriage is common (89). GBS isolates were serotyped by latex agglutination, and additionally subjected to multiplex PCR based for capsular polysaccharide genes. The GBS carriage rate was 12.0% (95% CI 9.4–15.0). Serotypes, Ia, Ib, II, III, IV, V, VI and VII were identified; serotype II was predominant (89). This was one of the first studies of GBS carriage to be performed in a rural SEA population, providing data of GBS in this region, the results from this study potentially have implications for the development of GBS vaccines (89).

1.8 The diagnosis of neonatal sepsis

In CH1, we use the criteria suggested by the expert meeting on neonatal and paediatric sepsis of European Medicines Agency (EMA) in 2010 for the diagnosis of probable sepsis

and culture-confirmed sepsis in neonates (90). Neonatal sepsis was defined by the EMA experts as the presence of at least two clinical criteria and at least two laboratory criteria in presence of or as a result of suspected or proven infection (frequently by identifying bacterial bloodstream infection) in neonates (90). A diagnosis of probable sepsis is made when the neonate has ≥2 clinical and ≥2 laboratory criteria of sepsis (90). The patient is latterly diagnosed with culture-confirmed sepsis when there is ≥1 positive blood culture of a pathogen (90).

The clinical criteria of neonatal sepsis suggested by EMA include abnormal body temperature (core temperature >38.5°C or <36°C and/or temperature instability); cardiovascular instability (bradycardia [mean heart rate <10th percentile for age in the absence of external vagal stimulus, beta-blockers or congenital heart disease or otherwise unexplained persistent depression over a 0.5–4h time period] or tachycardia [mean heart rate >2SD above normal for age in the absence of external stimulus, chronic unexplained persistent elevation over a 0.5–4h time period] and/or rhythm instability, reduced urinary output (<1 mL/kg/h), hypotension (mean arterial pressure <5th percentile for age), mottled skin, impaired peripheral perfusion); respiratory instability (apnoea episodes or tachypnoea episodes [mean respiratory rate >2SD above normal for age] or increased oxygen requirements or requirement for mechanical ventilation); gastrointestinal abnormalities (feeding intolerance, poor sucking, abdominal distension); skin and subcutaneous lesions (petechial rash, sclerema); and non-specific signs (irritability, lethargy, hypotonia) (90).

The laboratory criteria of neonatal sepsis suggested by EMA include white blood cells <4,000/mm^3 or >20,000/mm^3; immature to total neutrophil ratio (I/T) >0.2; platelet count

<100,000/mm^3; C-reactive protein (CRP) >15 mg/L; glucose intolerance (hyperglycaemia [blood glucose >180 mg/dL] or hypoglycaemia [blood glucose <45 mg/dL]); and metabolic acidosis (base excess < –10 mEq/L or serum lactate >2 mmol/L) (90).

1.9 The treatment of neonatal sepsis

Most guidance for the management of neonatal sepsis have been developed in high-income countries (91–93). While these guidelines are invaluable in describing optimal care, they are difficult to transpose to the complex clinical settings and less well-resourced healthcare facilities found in LMICs (94–96). Moreover, insufficient clinical assessments and a delay in the identification of sepsis cases impact on current management strategies.

A 12-month observational cohort study by the Neonatal AMR research network (NeoAMR) in 39 neonatal units from 12 LMICs found that ampicillin and gentamicin were the most common empirical antimicrobials for EOS; while cephalosporins, vancomycin, and amikacin were the usually used therapy for LOS (97). In SEA, empirical antimicrobial therapy is usually initiated with ampicillin and gentamicin (58). A study was conducted to describe the pattern of antimicrobial use of neonatal sepsis cases admitted to the NICU in the Hospital of Universiti Sains in Malaysia (54). All patients received empiric antimicrobial therapy, in which crystalline penicillin G plus gentamicin was the most commonly used therapy (69.4%) (54). When the infecting bacteria has been isolated and the clinicians have the results of antimicrobial susceptibility tests, the most appropriate secondary antimicrobial therapy should be given to the patient (98).

In clinical practice at CH1, empirical antimicrobial therapy is initiated immediately when an infectious condition is suspected. Prompt empirical treatment of neonatal sepsis with

broad-spectrum antimicrobials without waiting for blood culture result and on the basis of clinical manifestation and laboratory findings should be associated with an increased proportion of positive outcomes. Due to our local guidelines at CH1, the antimicrobials are stopped only when patients are in stable condition and acceptable or normal results of septic laboratory findings are demonstrated.

At CH1, the local management guidelines of sepsis in neonates recommend that the appropriate use of antimicrobials (indications, contraindications, doses, route of administrations, and side effects) must be guaranteed. Doses must be adjusted based on the function of liver and kidney and the duration of antimicrobial therapy must be warranted to have enough effectiveness. Antimicrobials are selected based on their concentration in the blood and at specific foci or sources of infections. Optimally, the choice of antimicrobials is based on the isolated bacterial pathogens from blood or another biological samples and their antimicrobial susceptibility patterns.

For EOS, the empirical antimicrobial regime at CH1 includes ampicillin, gentamicin, and cefotaxime. If the presentation is not severe, the initial regimen currently includes ampicillin and gentamicin. If the septic appearance is severe or the meningitis is suspected, a combination of ampicillin, gentamicin and cefotaxime is administered.

The use of antimicrobials for LOS at CH1 depends on whether the diagnosis is community-acquired sepsis (CAS) or hospital-acquired sepsis (HAS), both can happen after 72 hours of age in neonates. For CAS in neonates admitted to CH1, the empirical drugs of choice are based on the local bacterial profile and are generally a combination of cefotaxime and gentamicin. When *Staphylococcus* spp. is suspected, such as having

central catheters, invasive drainages, skin or umbilical infections, the local recommendations for CAS are oxacillin and gentamicin with or without cefotaxime. For HAS in neonates admitted to CH1, if the patient does not have a critical condition, we initially use ticarcillin, cefepime, or quinolones with or without amikacin; but if the neonate has severe sepsis or septic shock, we empirically use imipenem/meropenem or even with colistin for Gram-negative bacteria and vancomycin for Gram-positive bacteria to cover a wide range of bacterial pathogens that may trigger an increasingly serious condition.

At CH1, the duration of the antimicrobial course is 5–10 days for local infections (eye, skin, urinary tract infections), 10–14 days for sepsis or pneumonia, 21–28 days for severe sepsis, Gram-negative sepsis or meningitis. At CH1, we do not use aminoglycosides for >5–7 days due to their nephrotoxicity and ototoxicity in neonates.

During the course of antimicrobial therapy, the septic condition of patients must be monitored and evaluated closely. We routinely perform a sepsis panel including a complete blood count, C-reactive protein (CRP) and blood culture after 2–5 days of antimicrobial regime to make a clinical decision for the next steps of therapy. If the condition of patient becomes worse or does not resolve after 2–5 days and/or the laboratory panel shows abnormal values, then the antimicrobial therapy will be changed.

A change in antimicrobial at CH1 is dependent on the microbiological result including isolated pathogen and its antimicrobial susceptibility profile. However, in many cases when a microbiological result is not available, the antimicrobial is changed with an assumption about the possible pathogen. If a Gram-negative bacterium is suspected,

ticarcillin, quinolones (ciprofloxacin, pefloxacin, levofloxacin) or cefepime with or without amikacin are prescribed. If these drugs do not show effectiveness then infection with MDR Gram-negative bacteria, where upon imipenem, meropenem and colistin can be considered. Vancomycin is used when *Staphylococcus* spp. is suspected. Anaerobic bacteria are treated using metronidazole or clindamycin. The most common bacterial pathogens in neonatal sepsis and the appropriate antimicrobial (9,12,61,93,99,100) are shown in Table 1.5.

Table 1.5 Antimicrobial choices for common pathogenic bacteria

Pathogenic bacteria	**Antimicrobial choices** (9,12,61,93,99,100)
GBS	penicillin G or ampicillin
Listeria monocytogenes	ampicillin and gentamicin
Escherichia coli	cefotaxime or ampicillin and gentamicin
CoNS	vancomycin
Staphylococcus aureus	oxacillin or vancomycin
Enterococcus	ampicillin or vancomycin and gentamicin
Klebsiella, Serratia	cefotaxime or cefepime or imipenem or meropenem and gentamicin
Enterobacter, Citrobacter	cefepime or imipenem or meropenem and gentamicin
Pseudomonas aeruginosa	ceftazidime and gentamicin
MDR bacteria	cefepime or ticarcillin or imipenem or meropenem
Anaerobic bacteria	metronidazole or clindamycin

1.10 Antimicrobial resistance (AMR) in neonatal sepsis

An effective strategy for neonatal sepsis management in developing countries is of high relevance to reduce mortality and morbidity rate of new-borns in this region. Appropriate and standardized antimicrobial regimes are currently being examined in many clinical studies (95,96), but decreasing of antimicrobial susceptibility is a real threat to empiric antimicrobial strategies. Antimicrobial susceptibility data from organisms associated with BSIs in neonates are limited, but the susceptibility to first-and second-line antimicrobials in organisms associated with HAS have significantly decreased in developing countries (95,96).

A web-based survey of neonatal sepsis in 39 neonatal units in LMICs participating in the Neonatal AMR research (NeoAMR) network revealed alarming AMR trends among both Gram-negative bacteria (cephalosporin resistance 26%–84% and carbapenem resistance 0%–81%) and Gram-positive bacteria (vancomycin resistance 0%–45%) (97).

Ampicillin is the first choice antimicrobial for the treatment of EOS in neonates (12); however, Anderson and colleagues showed that only 18% of *Escherichia coli* isolates were susceptible to ampicillin (58), and other Gram-negative bacteria including, *Klebsiella* spp., *Enterobacter* spp., *Acinetobacter* spp., and *Pseudomonas* spp. were also commonly resistant to this antimicrobial (63%) (56). Resistance to gentamicin was also found to be significant, with a 30% to 37% resistance prevalence in Gram-negative bacteria resistant to this antimicrobial in the studies of Tiskumara (86) and Al-Taiar (30), respectively. These, and many other studies, show that the guidelines for initial use of antimicrobials in neonatal sepsis should be determined by common antimicrobial susceptibility patterns at local institutions in SEA. The work of Litzow and colleagues in

2 NICUs in the Philippines, found that 67% of Gram-negative organisms were resistant to ceftazidime (56). Resistance to amikacin, a commonly used aminoglycoside, was also identified in the study of Litzow with 52% of all Gram-negative bacteria being resistant (56). Furthermore, it was recognised by Tiskumara *et al*. that even when a third generation cephalosporin (frequently cefotaxime) was combined with gentamicin, that co-resistance was still common, with 56% of all Gram-negative bacteria being resistant to both agents (86). Consequently, many clinicians in SEA now rely on carbapenems, which are widely considered as "last resort" antimicrobials for Gram-negative bacteria. Carbapenems are now widely used and clinically valuable, but resistance to imipenem, the most regularly used carbapenem, was has also been detected, and ranged from 5.7% to 20% in the studies of Ariffin (101) and Litzow (56), respectively.

Concerns regarding AMR in neonatal sepsis have recently been refreshed in SEA. This concern is supported by many sepsis cases associated with MDR organisms, which are dominated by the Gram-negative bacteria, *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii* in this global AMR "hot spot" (58,102). In a study comparing multiple features of nosocomial infection in an NICU of both Kuala Terengganu Hospital (HKT) and Universiti Sains Malaysia Hospital (HUSM) in 1998, half of *Klebsiella pneumoniae* septic cases were resistant to cephalosporins and aminoglycosides in HKT; a similar proportion of *Klebsiella aerogenes* isolates were resistant to piperacillin and aminoglycosides in HUSM (102). A further prospective cohort study of Al-Taiar in neonatal care units in Malaysia and Thailand found that 47%, 37% and 32% of all Gram-negative organisms were resistant to third generation cephalosporins, gentamicin or both, respectively (30). Research concerning the antimicrobial susceptibilities of bacteraemia isolates in infants in Laos from 2000 to 2011 found that the most common pathogens

isolated during this study were *Staphylococcus aureus*, *Escherichia coli,* and *Klebsiella pneumonia* (58). No methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated, but 82% of *Escherichia coli* were resistant to ampicillin (58). In Gram-positive bacteria, particularly *Staphylococcus aureus*, susceptibility to vancomycin was well preserved (58). The main findings of AMR patterns in organisms associated with neonatal sepsis in SEA are outlined in Table 1.6.

Table 1.6 Antimicrobial resistance patterns in neonatal sepsis in SEA

Antimicrobial resistance patterns		AMP	3GC	GEN	3GC+GEN	AMK	IPM	SXT	VAN
Tiskumara R	Gram-negative bacteria		30.0%	30.0%	56.0%				
Wan Hanifah W	*Klebsiella pneumoniae*		50.0%	50.0%		50%			
Litzow JM	*Klebsiella, Enterobacter, Acinetobacter* and *Pseudomonas* spp.	63.0%	67.0%			52.0%	20.0%	41.0%	
Ariffin N	Gram-negative bacteria		36.1%			8.8%	5.7%		
Al-Taiar	Gram-negative bacteria		47.0%	37.0%	32.0%				
Anderson M	*Escherichia coli*	82.0%							
Anderson M	*Staphylococcus aureus*								0.0%

AMP: ampicillin, 3GC: third generation cephalosporins, GEN: gentamicin, AMK: amikacin, IPM: imipenem, SXT: trimethoprim-sulfamethoxazole, VAN: vancomycin (30,56,58,86,101,102)

1.11 Adjunctive therapies for the treatment of neonatal sepsis

Pentoxifylline (PTX), which is a methylxanthine derivative, has been found to inhibit the release of pro-inflammatory cytokines and has other effects on the immune system (103). In Malaysia, the effectiveness of PTX on plasma tumour necrosis factor (TNF) alpha and interleukin (IL)-6 in neonatal sepsis was evaluated in a study of 20 neonates (104). The results showed that pentoxifylline had no effect on leukocyte counts, serum CRP levels, TNF alpha, IL-6 levels or mortality in neonates with sepsis (104).

Further, a double-blinded randomized controlled trial of glutamine-enriched neonatal parenteral nutrition was conducted in the Paediatric Department, Universiti Sains in Malaysia to measure the incidence of clinical-proven sepsis and blood culture-proven sepsis in neonates. The incidence of clinical-proven sepsis and blood culture-proven sepsis was not significantly different in the intervention group (adding glutamine to the parenteral nutrition) and the control group (receiving standard parenteral nutrition) (15.7% vs. 10.2%, p=0.21 and 16.5% vs. 15.7%, p=0.38, respectively) (105). The study found no benefit of the addition of glutamine to neonatal parenteral nutrition for reducing the incidence of sepsis in neonates (105).

The effectiveness of intravenous immunoglobulin in sepsis was evaluated in a study with 102 neonates at the Children's Hospital, Bangkok, Thailand from February 1988 to February 1990 (106). The study found that, in comparison to a control group (not given immunoglobulin), that those given intravenous immunoglobulin had significantly lower infection and mortality rates (106). The authors also observed that an immunoglobulin dose of 250 mg/kg was effective as well as the dosage of 500 mg/kg (106).

1.12 Prevention of neonatal sepsis and infection control

A number of strategies has been developed to prevent and control the neonatal sepsis in SEA (107–109). The balanced scorecard (BSC), an executive performance measurement consisting of four perspectives (increasing the knowledge-attitudes-skills of employees; implementation of prevention and infection control practices; decline of BSIs, mortality rate, length of stay, and hospitalization cost; improving customer satisfaction) was conducted at the neonatal unit of Cipto Mangunkusumo Hospital in Indonesia (107). This BSC was found to reduce the prevalence of BSIs from 5.23% to 0.13% in neonates with a birth weight of 1,000–1,499 g, and from 2.99% to 0.16% in neonates with a birth weight of 1,500–1,999 g (107). In this setting, the source of the infectious organisms was considered to be the environment, such as tap water and humidifying water in the incubator (107).

Data from the NICU from the Women and Children's Harapan Kita Hospital in Jakarta, Indonesia also showed that a closed system of the intravenous fluid infusion dramatically reduced the incidence of sepsis on days 3–5 with 37 cases in the first period compared to 5 cases in the second period (108). Therapeutically, crystalline penicillin plus gentamicin prescribed within 24 hours of life was found to be effective in the prevention of EOS in neonates at a Malaysian government hospital (109). However, low birth weight neonates had a higher risk of treatment failure (109).

Although the attitude, knowledge and practice of prevention and infection control have been gradually improved at CH1, safeguarding of an aseptic neonatal environment remains a challenge as prolong use of vascular lines, parenteral nutrition, and respiratory support cannot be avoided in a variety of clinical conditions in neonates. In a study by

Lee and colleagues, a prior long line was demonstrated to be associated with first septicaemia episode (OR=7.07; 95% CI 1.37–36.47 with *p*=0.019) but not prior assisted ventilation (52). The risk factors for *Burkholderia cepacia* were contaminating water in an oxygen humidifier, ventilator water traps, and a humidifier water trap (52).

A nosocomial outbreak of *Enterobacter gergoviae* was identified in a study by Ganeswire (110). The source of infection from this study was likely to have been dextrose saline in parenteral antimicrobials and the hands of a healthcare providers (110). Pulsed-field gel electrophoresis of Xba I-digested chromosomal DNA in this study then confirmed the potential of cross-contamination of parenteral dextrose saline and the healthcare providers (110). *Pantoea* spp. infection was detected in 8 neonates receiving parenteral nutrition; seven of which died. Environmental sampling and parenteral fluids from the NICU and the pharmacy were cultured during the outbreak. Parenteral nutrition was the cause and the contamination may occurred during the preparation in the pharmacy (111). Compliance with standard diagnosis and treatment guidelines must be guaranteed for infection prevention and control strategies. A minute-by-minute observational assessment after a number of deaths due to nosocomial infections in a Philippines hospital revealed that performance and timing of practices and interventions in new-born care including drying, kangaroo mother care, delayed cord clamping, breastfeeding and delayed bathing were all at lower standards than recommended by WHO guidelines (59).

1.13 Factors associated with the outcome from neonatal sepsis

There are many factors contributing to the outcome of neonatal sepsis, however, the exact clinical predictors of poor outcomes in LMIC settings are poorly characterised and exacerbated by limited or non-existent local surveillance systems (95,96). From research

conducted in the Philippines, the case fatality rate was higher in neonates <1 week of age, or with dense or diffuse infiltrates in chest X-ray (53). Meningitis or meningoencephalitis and *Klebsiella pneumoniae* infection were independently associated with mortality in a retrospective study in Cambodia from January 2007 to July 2011 (29). Mortality was found to be the highest in neonates with Gram-negative BSIs compared with no confirmed bloodstream infection and Gram-positive bloodstream infection in a prospective study of all positive blood cultures among neonates during a 12-month period in southern Vietnam (26). A case-control study in a Thailand indicated that mortality in carbapenem-resistant *Acinetobacter baumannii* group (n=14) was higher than those infected with carbapenem-susceptible *Acinetobacter baumannii* group (n=38) (42.9% vs. 13.2%; OR=5.0; p=0.02) (98).

There are limited current data linking the clinical features of bacterial neonatal sepsis with the factors associated with mortality in Vietnam. Understanding these factors is essential if we are to identify the most "at-risk" patients early, in order to plan strategies to improve outcome. Early recognition of warning signs would allow more timely evaluation, recognition of at-risk patients, the development of a risk-based management approach and more effective treatment.

1.14 Neonatal disease severity scoring systems

Illness severity scoring systems are often used in neonatal populations for different purposes such as, 1) evaluating the severity of the disease, the mortality risk, and short-term and long-term outcomes, 2) to assess the resource utilisation and compare the quality management between various institutions, 3) to serve as control for population variables, particularly important for performing clinical trials (112). Multiple score systems have

been designed to assess the severity of disease in neonates. Each score has its own strengths and limitations; therefore, none of these systems have been found to be ideal (112). The Neonatal Therapeutic Intervention Scoring System (NTISS) has been used to evaluate the severity of illness in neonates requiring intensive care (113). NTISS was originally created based on treatment and interventions, with points assigned from 1 to 4 for various therapies rather than on pathophysiological parameters, such as the Clinical Risk Index for Babies (CRIB II), Score for Neonatal Acute Physiology (SNAP), and Score for Neonatal Acute Physiology-Perinatal Extension (SNAPPE) (114,115). The NTISS scores have been used to compute the total score of interventions within the first 24 hours after admission. It was found that this scoring system can be used as an indicator of neonatal illness severity and resource utilization (113). In addition, the predictive performance of NTISS score, evaluated 24 hours after admission for mortality in term and preterm neonates, provided the same precision for both VLBW and ELBW neonates (113,116,117). For these reasons, I selected the NTISS for evaluating the severity of the disease in neonates with sepsis. The details of NTISS including respiratory and cardiovascular parameters, monitoring, drugs, metabolic/nutrition, procedures, transfusion, and vascular access (113) can be found in Appendix 9.8.

1.15 Neonatal sepsis in high-income settings

In the United States, the incidence of neonatal sepsis increases with the decrease of gestational age. The incidence of EOS in the United States has decreased largely due to reduction in GBS infections as a result of intrapartum antibiotic prophylaxis (IAP) (118–120). In term neonates, the incidence of neonatal sepsis including both EOS and LOS is 1–2 cases per 1,000 live births (14,121). In a prospective national surveillance study between 2006 and 2009 in the United States, the incidence of EOS was 0.98 cases per

1,000 live births; the rate among neonates with birth weight >2,500 g was 0.57 per 1,000 (46). In late preterm neonates, the incidence of neonatal sepsis is higher than in term neonates with the reported incidences of EOS and LOS in late preterm neonates were 4.4 and 6.3 per 1,000, respectively (122). In term of EOS, GBS and *Escherichia coli* are the most common bacterial aetiologies from data of the NICHD registry in which GBS (43%, 0.41 per 1,000 live births) and *Escherichia coli* (29%, 0.28 per 1,000 live births) were the most frequently isolated pathogens (46). In term of LOS, from data of the NICHD study of VLBW neonates with sepsis, nearly half of the aetiologies were CoNS (48%); followed by 22% of other Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus*, GBS), and 18% of Gram-negative bacteria (*Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, and *Serratia*) (17).

In the United Kingdom, the incidence of all infections including CoNS in neonatal population was 8 per 1,000 live births and 71 per 1,000 neonatal admissions between 2007 and 2008 (22). These infections mostly occurred in premature (82%) and low birthweight (81%) neonates (22). The incidence of EOS was 0.9 per 1,000 live births and 9 per 1,000 neonatal admissions (22). GBS (58%) and *Escherichia coli* (18%) were the most common isolated bacteria in EOS (22). The incidence of LOS was 7 per 1,000 live births and 61 per 1,000 neonatal admissions including CoNS. CoNS (54%), Enterobacteriaceae (21%) and *Staphylococcus aureus* (18%) were the most common isolated organisms in LOS (22).

1.16 Clinical impact of AMR

The first estimate of neonatal mortality as a consequence of AMR was published in 2016 with MDR bacteria accounted for approximately 30% of all global neonatal deaths caused by sepsis (123). MDR bacteria are been known as a challenge in high-income countries,

but the situation is much worse in LMICs, due to unrestricted access to antimicrobials, a higher burden of infectious disease, weaker medical systems, and resources shortage (124).

Currently, MDR Gram-negative bacteria raise utmost concern in the neonatal population, as limited alternative therapeutic antimicrobials are available (124). Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae and carbapenem-resistant Enterobacteriaceae (CRE) are increasingly associated with outbreaks of HAS in NICUs, and correlate with considerable morbidity and mortality (7). The emergence of ESBL-producing Enterobacteriaceae and CRE among NICUs has unsurprisingly led to the reinstatement of older antimicrobials or the usage of antimicrobials with minimal information on effectiveness, and optimal dosing (7).

Neonatal sepsis caused by MDR Gram-negative bacteria is associated with poorer outcomes, including significantly higher proportions of infectious complications and overall mortality (125). A case-control study conducted in Taiwan between 2001 and 2012 reported that 14.2% of LOS caused by Gram-negative pathogens were due to ESBL-producing organisms (62.3% of *Klebsiella* spp., 20.8% of *Escherichia coli*, and 16.9% of *Enterobacter* spp.) (125). In this study, neonatal sepsis caused by ESBL-producing Gram-negative bacteria were associated with a higher proportion of infectious complications (p=0.008) and adverse outcomes (p=0.049), compared to cases with non-ESBL producing organisms (125).

A prospective longitudinal study conducted in 17 Australasian neonatal centres between 1992 and 1994 investigated the incidence and outcome of methicillin sensitive (MSSA)

and methicillin resistant *Staphylococcus aureus* (MRSA) sepsis in neonates (126). The neonatal mortality of MRSA sepsis was 24.6% compared to 9.9% of MSSA sepsis (126). The mortality of EOS caused by MSSA was 39% (7 of 18), compared to 7.3% of LOS caused by MSSA (126).

Available data for the ideal treatment of MDR associated sepsis in children and in new-born infants are inadequate, with most data arising from retrospective observational studies (7). Data of some clinical studies assessing old or new antimicrobial therapies relating to neonates show that less than ten antimicrobial trials have been performed on premature neonates globally (124,127).

1.17 Neonatal sepsis in Southeast Asia

Sepsis remains a leading cause of neonatal hospital admission, morbidity, and mortality in LMICs (128). In LMICs, bacterial infection, including bacteraemia, is complicated by MDR organisms, particularly when related to healthcare acquired infection, and effective management of neonatal sepsis is increasingly problematic (128). Recently, the WHO has acknowledged the problem of AMR as an endemic and widespread problem in LMICs (129). In many LMICs untreatable bacterial infections with broadly AMR pathogens are no longer a threat but a common reality. AMR in LMICs represents one of the biggest threats to global health and is one of the greatest current challenges in infectious disease research.

While AMR is an issue with all types of bacterial infection, the issue is most acute in management of clinical sepsis. This is a particular problem in neonates, due to high mortality/morbidity rates and the timely need for rapid detection and treatment of the

causative pathogen. Sepsis demonstrates extensive geographical diversity in both aetiology and proportions of AMR bacteria isolated (130,131). Understanding the local and regional epidemiology of sepsis in hospitalized neonates is crucial in the development of rational management and treatment guidelines, especially in high-risk AMR LMICs like Vietnam.

1.18 Sepsis and AMR in Vietnam

The incidence of AMR BSIs in Vietnam has increased over recent years, and is predicted to increase further (132). This trend has is induced by an increase in both Gram-negative and Gram-positive pathogens, chiefly *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus,* and *Enterococci* (132).

The aetiology of community-acquired BSIs in Vietnam has changed considerably over the 20 years. In Vietnam, we reported that the antimicrobial resistance in *Salmonella* Typhi severely restricted the options available for antimicrobial treatment (133). A previous study documented the decline of *Salmonella* Typhi (the predominant pathogen isolated from blood) in Vietnam from 2002 and the subsequent increase in non-typhoidal *Salmonella* and other opportunistic HIV-associated pathogens (134). This shift is likely to reflect a changing landscape of infectious disease related to the HIV epidemic, urbanization, and secondary social determinants within Vietnam. Vietnam, as with many countries in Asia, is undergoing a rapid economic transition, and programmes to improve sanitary conditions have reduced the overall risk of water-borne infections. HIV-associated opportunistic pathogens have now emerged as the leading cause of BSIs and the primary cause of mortality in hospitalized adult patients in this location. These studies were performed at the Hospital for Tropical Diseases in Ho Chi Minh City and thus

included mainly adults, including those with HIV, and children but not neonates (135–137). Therefore, these observations may not be fully representative of the situation in children or neonates.

1.19 Knowledge gaps

Little is known about contemporary antimicrobial susceptibility patterns and their underlying genetic determinants in the major causes of neonatal bacterial of sepsis in Vietnam. Furthermore, the impact of AMR and clinical factors on disease progression and outcome in neonates in LMICs is also not well documented. To address these issues, here, I aimed to investigate the aetiology of pathogens associated with bacterial sepsis in neonates and to detail the effects of reduced antimicrobial susceptibility on the outcome of sepsis in neonates.

The studies conducted here were a clinical and microbiology research collaboration between Children's Hospital 1 (CH1) and the Oxford University Clinical Research Unit (OUCRU) in Vietnam. Theses studied will hopefully act as conduit for introducing molecular biology for bacteriology into routine hospital care into CH1 and will lead to future studies investigating appropriate empirical treatment for bacteraemia and the impact of AMR on the outcome of sepsis in the paediatric and neonatal population in Vietnam.

1.20 Rationale and hypothesis

There are limited contemporary data on the causes of bacterial sepsis in neonates in Vietnam. To understand the causes of neonatal sepsis and to best inform antimicrobial treatment regimes in our setting I performed a prospective observational study at

Children's Hospital 1 in Vietnam from January 2017 to June 2018 (138). I hypothesized that there have been recent increases in MDR Gram-negative bacteria causing sepsis in hospitalised neonates, and that MRSA has emerged as an important pathogen, and that factors associated with AMR impact on disease outcome.

1.21 Aims and objectives

I aimed to investigate the clinical features, major bacterial causes of neonatal and childhood sepsis, their antimicrobial susceptibility patterns, and some genomic profiles of isolated bacteria and their association with disease outcomes. My study objectives were:

- To retrospectively determine the longitudinal time trends in the profile of bacteraemia-associated pathogens isolated in children of all ages and their antimicrobial susceptibility patterns at CH1 in HCMC from 2013 to 2016, specifically related to the emergence of AMR organisms.
- To prospectively describe the clinical characteristics including demographic features, clinical signs and symptoms, diagnoses, associated conditions, treatment, and outcomes of neonates with sepsis at CH1 in HCMC from January 2017 to June 2018.
- To study the bacterial profile and the antimicrobial susceptibility of bacteria associated with neonatal sepsis.
- To analyse a collection of genomics associated with *Acinetobacter baumannii* causing neonatal sepsis.
- To determine the clinical, microbiological, and genomic factors associated with the outcome of neonates with sepsis.

2 Materials and methods

2.1 General methods

2.1.1 Ethics, regulatory approvals, and governance

The work for this thesis was constructed around 4 main components. 1) An observational study to retrospectively describe the microbiology of bacteria isolated from the blood culture of children between 2013–2016 in CH1 in HCMC; 2) A prospective study to investigate the clinical features of sepsis in neonates from January 2017 to June 2018 in CH1 in HCMC; 3) to describe the aetiological agents and their corresponding antimicrobial susceptibility of neonatal sepsis from January 2017 to June 2018 in CH1 in HCMC; and 4) to investigate the genomes of *Acinetobacter baumannii* causing neonatal sepsis from January 2017 to June 2018 in CH1 in HCMC.

This study contributing the data for this work was sponsored by the University of Oxford and was monitored by the Clinical Trials Unit at Oxford University Clinical Research Unit (OUCRU). This study was conducted in accordance with the principles of the Declaration of Helsinki and the terms of approval of the appropriate ethical committees (139). This study was conducted in full conformity with relevant regulations and with the International Council on Harmonization Guidelines for Good Clinical Practice (140).

This study had the approvals of the Oxford Tropical Research Ethics Committee (OxTREC) and the institutional review board (IRB) of CH1. The ethics reference numbers are Oxford, England (OxTREC 35-16) (Appendix 9.1) and Vietnam (CH1 Ethics Committee 122/GCN-BVND1) (Appendix 9.2). Trial registration number of this study is

ISRCTN69124914 (Appendix 9.3). The study protocol can be found in Appendix 9.4. The protocol has been published previously (138) and this paper is available in Appendix 9.9.

2.1.2 Participant risks, expenses, and confidentiality

This was a minimal risk study because it did not involve any investigational new drugs or interventions. The collection of all biological samples for use in this study was performed as part of routine clinical assessments and were consistent with the local standard of care and good clinical practice. Patients did not have to pay any expenses to participate in this study. Patients did not receive reimbursement of expenses. All data were stored securely in password protected devices. All specimens, reports, study data collection, process, and administrative forms were identified by a coded number. Study databases were secured with password-protected access systems.

2.1.3 Informed consent

Trained, GCP-accredited members of the study team collected informed consent. The team discussed the study with the accompanying parent/guardian. If both parents were deceased or not actively involved in child care, the main long-term carer of the child was accepted as the guardian and considered able to give consent for the study. Study staff described the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants, and alternatives to enrolment. The parent/guardian was invited to ask questions, which were addressed by study staff, and they were provided with appropriate contact numbers if having any subsequent questions. If the parent/guardian agreed for the child to participate, they were asked to sign and date an informed consent form. A copy of the patient information sheet and the informed consent form were given to them to keep. In addition to the procedures above, illiterate signatories

had the informed consent form read to them in the presence of a witness who would sign to confirm this. The participant's information sheet (Appendix 9.5) and consent form (Appendix 9.6) were written in the local language with terms that were easily understandable.

2.1.4 Data management

The investigator was responsible for maintaining all study records. The investigator was responsible for the timeliness, completeness and accuracy of the information in the original dataset and the clinical data management system. CH1/OUCRU-VN staff entered data directly onto computer-based data collection programs which were uploaded securely to an Internet-based database. Laboratory staff recorded specimens (and their aliquots), their storage location, and their shipments. All necessary tools, instruction, and training were provided to all site staff involved in data entry to ensure the correct and consistent completion database prior to the study starting.

2.1.5 Quality control and quality assurance procedures

The study was conducted in compliance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. Regular monitoring was performed according to ICH GCP. Data, samples and procedures were evaluated for compliance with the protocol, standard operating procedures, regulatory requirements and terms of ethical approval. Records were verified for accuracy against source documents and physical inventory of samples. The diagnostic laboratory at CH1 and OUCRU conducted regular internal and external quality control procedures.

2.1.6 Sample use and storage

Use of stored human specimens

No human samples were used in this study; this study only investigated the bacteria cultured from those with a bacterial bloodstream infection.

2.1.7 Study setting

HCMC (formerly known as Saigon) is the largest city in the southern part of Vietnam (Figure 2.1) with an estimated population of 8.4 million in 2016; 23.8% of the population are children aged 0–14 years (141).

Figure 2.1 Location of Ho Chi Minh City in Southeast Asia

Ho Chi Minh City is located in the south-eastern region of Vietnam and is the largest and most populated city in Vietnam (141).

CH1 is the largest tertiary paediatric centre in HCMC and in southern Vietnam with 1,400 inpatient beds and >1,600 staff. CH1 receives ~1.5 million outpatient visits and 95,000 admissions each year, care is provided to all children <15 years old from HCMC and other provinces of southern Vietnam. The hospital was built and put into operation in October 1956. CH1 is considered to be a key children's hospital in the region with clinical departments covering all subspecialties of paediatrics including nephrology, haematology and haemorrhagic Dengue fever, neonatology, cardiovascular diseases, emergency and intensive care units, gastroenterology and hepatology, neurology and infectious diseases, respiratory diseases, and nutrition. The clinical development strategy of CH1 is to focus on four priorities including 1) neonatology, 2) emergency and intensive care medicine, 3) surgery (particularly heart surgery and surgical repair of congenital abnormalities), and 4) infectious diseases.

The neonatal centre at CH1 (Figure 2.2) was established in 2005 and was the first neonatal centre in Vietnam. The centre has 120 inpatient beds in the neonatology department with an additional 30 beds in the NICU, supported by a total of 27 neonatologists and 130 nurses. Advanced clinical techniques such as high frequency oscillatory ventilation and non-invasive respiratory support, inhaled nitric oxide for pulmonary hypertension, whole body cooling for hypoxic ischemic encephalopathy, continuous renal replacement therapies, interventional cardiology, heart surgery, surgical repair of congenital defects, and intensive care of extremely low birth weight and extremely premature neonates are routinely performed.

Figure 2.2 The Neonatal centre at CH1

Some photographs of staff members working and the medical facilities at the neonatal centre at CH1. These photographs aim to help the reader contextualise the studies presented.

OUCRU is based within the Hospital for Tropical Diseases (HTD) in HCMC, a tertiary referral hospital for infectious diseases of southern Vietnam, under the management of the Department of Health of HCMC and the Vietnam Ministry of Health. OUCRU has a large clinical and scientific research programme which focuses on the most significant infectious diseases in Vietnam. The OUCRU laboratory capacity covers multidisciplinary subspecialties including immunology, bacteriology, virology, genetics, genomics, metabolomics, mathematical modelling, bioinformatics, biostatistics, health economics, and epidemiology.

2.2 Methods for retrospective study

2.2.1 Ethical considerations for retrospective study

This retrospective study had ethical approvals from OxTREC (OxTREC 35-16) and the research ethical committee of CH1 (122/GCN-BVND1). Additionally, permission from CH1 was granted to use microbiological information of BSIs in databases between 2013 and 2016 for purposes of research. Data acquired from the retrospective study and details of patients were anonymised. Informed consent from patients in a retrospective study was not required.

2.2.2 Study site

I retrospectively conducted an observational study to characterize the organisms and their corresponding antimicrobial susceptibility patterns isolated from blood culture of children of all ages at CH1 in HCMC in Vietnam from 2013 to 2016.

2.2.3 Study design

This was a retrospective observational study. I extracted available microbiological data including the identification and the antimicrobial susceptibility patterns of bacteria isolated from the blood culture of children of all ages admitted to CH1 in HCMC in Vietnam from 2013 to 2016. The identification and the antimicrobial susceptibility data of isolated bacteria were stored in the departmental computer housed in the microbiology laboratory of CH1 and in the form of Microsoft Excel files.

My main interest was to specifically study the microbiology of bacteria isolated from blood culture in the sepsis of neonatal population. However, to put these organisms into

the context of bacteraemia at CH1, I studied all cause bacteraemia data stored in the computer housed at the CH1 microbiology laboratory. The database included the identification of isolated bacteria in numbers of isolates and the percentage of AMR of these organisms to different types of antimicrobials from 2013 to 2016. In this chapter, I assessed the major causes of bacteraemia in children at CH1 and their antimicrobial susceptibility patterns.

2.2.4 General procedures of blood culture at CH1 between 2013 and 2016

Blood samples (1–2 mL) were inoculated into the BACTEC PEDS Plus ™/F blood culture bottle (Becton, Dickinson and Company, New Jersey, US) and placed into a BACTEC automatic blood culture system, such as BACTEC ™ 9120 and BACTEC ™ FX (Becton, Dickinson and Company, New Jersey, US). After 24–48 hours, if bacterial growth is recorded, the organisms are sub-cultured and subjected to identification and antimicrobial susceptibility testing using the VITEK 2 COMPACT automated machine (bioMérieux, Craponne, France).

Antimicrobial susceptibility testing was performed by the disk diffusion method (Oxoid Thermo Fisher Scientific Ltd, Basingstoke, Hampshire, UK) using guidelines established by the Clinical and Laboratory Standards Institute (CLSI) and, by minimum inhibitory concentrations (MICs) by VITEK 2 COMPACT automated machine (bioMérieux, Craponne, France).

2.2.5 Inclusion and exclusion criteria

The inclusion criteria were all data of bacterial isolates and their available antimicrobial susceptibility patterns from the blood cultures of hospitalised children at CH1 from 1st

January 2013 to 31st December 2016. There were no exclusion criteria. It was not practical to thoroughly investigate the epidemiology of BSIs, the demographics, and the clinical features of patients having positive blood cultures admitted to CH1 due to limited retrospective data.

2.2.6 Study size

As this was a retrospective descriptive study, the sample size was dependent on the number of bacteria isolated from blood culture with available data stored in the computer housed at the CH1 microbiology laboratory from 2013 to 2016.

2.2.7 Study procedures

Details including bacterial identification and antimicrobial susceptibility in BSIs of children of all ages were extracted from the available electronic database. It was not practical to obtain and review the hospital records of all patients with a positive blood culture episode to investigate to demographic, clinical, and outcome features; and to ascertain whether a pathogen was clinically relevant for that particular patient. The antimicrobials used for susceptibility testing varied between different bacteria and between years. Generally, the susceptibilities of Gram-negative organisms to gentamicin, trimethoprim-sulfamethoxazole, chloramphenicol, ampicillin, ticarcillin-clavulanic acid, cefuroxime, cefotaxime, cefpodoxime, ceftazidime, ceftriaxone, cefepime, nalidixic acid, ciprofloxacin, aztreonam, imipenem, meropenem, and ertapenem were measured. The antimicrobial susceptibilities of Gram-positive organisms to gentamicin, erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole, penicillin G, oxacillin, cefoxitin, ciprofloxacin, rifampicin, and vancomycin were also measured.

2.2.8 Quality control for the retrospective study

This retrospective study was conducted in compliance with the approved protocol, ICH GCP, relevant regulations, and standard operating procedures. Extracted databases were evaluated for compliance with the inclusion criteria that all available and essential data were collected. Regular monitoring was performed according to ICH GCP by the monitoring team of OUCRU. Extracted databases were considered to be the source documents and results of analysis were verified for accuracy against source documents. The microbiology laboratory at CH1 has regularly conducted internal and external quality control procedures for tests listed at http://www.boa.gov.vn/en/microbiology-laboratory-childrens-hospital-1 to receive the international ISO 15189:2012 accreditation, the Vietnamese quality TCVN 15189:2014 accreditation, and the Vietnam accreditation scheme for medical testing laboratory VILAS MED 019.

2.2.9 Data analysis

Data are presented in the form of tables and bar charts for descriptive analysis. Over the 4-year period from 2013 to 2016, the number and percentage of isolates and the antimicrobial susceptibility patterns were determined. The coverage of antimicrobial therapy was a theoretical assumption based on the lowest susceptibility proportions of isolated bacteria to major antimicrobials between 2013 and 2016. All data analysis was performed using Stata version 14 and R version 3.4.2.

2.3 Methods for the clinical study

2.3.1 Ethical considerations for prospective study

OxTREC (OxTREC 35-16) and the IRB of CH1 (CH1 Ethics Committee 122/GCN-BVND1) provided ethical approvals for the prospective study. This prospective observational study was a minimal risk study because it did not involve any new drugs or interventions. All procedures were performed as part of routine clinical practice and were consistent with the local standard of care and good clinical practice. Written informed consent was given by a parent or guardian. Patient identifying information was anonymised in publications or presentations resulting from this work. The Strengthening the Reporting of Observational Studies in Epidemiology for New-born Infection (STROBE-NI) recommendations were used as a standard for reporting in this study (142).

2.3.2 Study design

I conducted a prospective, observational study to characterise the clinical features, to measure mortality, and determine factors associated with mortality of neonatal sepsis at CH1 in HCMC in Vietnam. In this study, I recruited all patients who fulfilled the inclusion criteria and who were admitted to CH1 in HCMC in Vietnam from January 2017 to June 2018. The protocol for this study has been published previously (138) and is available in Appendix 9.4.

2.3.3 Sample size

In this prospective observational study, I recruited all patients who fulfilled the inclusion criteria and were admitted to CH1 in HCMC in Vietnam from January 2017 to June 2018. The sample size was dependent on the number of patients meeting the inclusion criteria over the designated time period. I recruited 524 participants during the study period. Blood cultures were performed in all cases.

2.3.4 Inclusion and exclusion criteria

Neonates (≤1 month of age) clinically diagnosed with a primary probable sepsis, who had a blood culture taken, and who were in-patients at CH1 were recruited into the study, after written informed consent was given by a parent or guardian. Patients were excluded when informed consent was not provided, the length of hospital stay was <24 hours, death was imminent within 12 hours, or the patient had been previously recruited in the study. Only the primary episode of sepsis was included for each of enrolled neonates, repeat episodes from the same neonate were excluded.

2.3.5 Study definitions

Here, I used the criteria suggested by the European Medicines Agency (EMA) in 2010 for the diagnosis of sepsis in neonatal population (90). Neonatal sepsis was defined by the EMA experts as the presence of at least two clinical criteria and at least two laboratory criteria in presence of or as a result of suspected or proven infection (frequently by identifying bacterial bloodstream infection) in neonates (90). The diagnosis of probable sepsis was made when the neonate had ≥2 clinical criteria and ≥2 laboratory criteria of sepsis. The patient was subsequently diagnosed as culture-confirmed sepsis when having ≥1 positive blood culture of a pathogen (90).

The clinical criteria for the diagnosis of neonatal sepsis (143) included: abnormal body temperature (core temperature >38.5^{o}C or <36^{o}C and/or temperature instability); cardiovascular instability (bradycardia [mean heart rate <10th percentile for age in the absence of external vagal stimulus, beta-blockers or congenital heart disease or otherwise unexplained persistent depression over a 0.5–4h time period] or tachycardia [mean heart rate >2SD above normal for age in the absence of external stimulus, chronic unexplained persistent elevation over a 0.5–4h time period] and/or rhythm instability, reduced urinary output (<1 mL/kg/h), hypotension (mean arterial pressure <5th percentile for age), mottled skin, impaired peripheral perfusion); respiratory instability (apnoea episodes or tachypnoea episodes [mean respiratory rate >2SD above normal for age] or increased oxygen requirements or mechanical ventilation requirement); gastrointestinal abnormalities (feeding intolerance, poor sucking, abdominal distension); skin and subcutaneous lesions (petechial rash, sclerema); and non-specific signs (irritability, lethargy, hypotonia) (90).

The laboratory criteria for the diagnosis of neonatal sepsis (143) included: white blood cells <4,000/mm^3 or >20,000/mm^3; platelet count <100,000/mm^3; serum C-reactive protein (CRP) >15 mg/L; glucose intolerance (hyperglycaemia [blood glucose >180 mg/dL] or hypoglycaemia [blood glucose <45 mg/dL]); and metabolic acidosis (base excess < –10 mEq/L or serum lactate >2 mmol/L) (90).

EOS was defined as the onset of sepsis ≤72 hours after birth (9,11–14). LOS was defined as sepsis developing >72 after birth (9,15–18). Hospital-acquired sepsis (HAS) was defined as sepsis occurring ≥48 hours after hospital admission (49). Community-acquired

sepsis (CAS) was defined as sepsis arising outside the hospital or <48 hours after hospital admission (144). Sepsis complicated by organ dysfunction was defined as severe sepsis (79,80). Septic shock was defined as the presence of cardiovascular dysfunction and tissue hypoperfusion (79,80). Empirical antimicrobial therapy was considered to be inappropriate if the treatment regimen did not include at least 1 antimicrobial that was active in vitro against the infecting microorganisms within 24 hours of blood culture. The neonatal sepsis attributable death certification was made based on the independent assessments of two qualified neonatologists and sepsis attributable death was recorded according to the neonatologists' presumption that any neonates who died after onset of sepsis.

The diagnosis of other infectious conditions (pneumonia, catheter-related bloodstream infection, necrotizing enterocolitis, meningitis, peritonitis, and urinary infection) and comorbidities (congenital heart diseases, congenital gastrointestinal anomalies, respiratory distress syndrome, bronchopulmonary dysplasia, pulmonary hypertension, congenital renal anomalies) were based on standard definitions for neonatal-perinatal medicine (1–3).

Prematurity was identified as gestational age <37 weeks and extreme prematurity as gestational age <28 weeks. Low birth weight was defined as birth weight <2,500 grams and extremely low birth weight referred to birth weight <1,000 grams (1–3).

2.3.6 Study procedures

I recorded and collected demographic, clinical and laboratory information of the patients including demographic data, maternal factors, clinical characteristics, laboratory results,

diagnoses, treatments, and outcomes. Patient data were collected on individual case report forms. Data from these records were subsequently entered into CliRes Data Management System of OUCRU. These were source data for this study.

2.3.7 Quality control for the prospective study

The prospective study was conducted in compliance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. Regular monitoring was performed according to ICH GCP by the monitoring team of OUCRU. Data, samples and procedures were evaluated for compliance with the protocol, standard operating procedures, regulatory requirements and terms of ethical approval. The diagnosis of neonatal sepsis was carefully discussed for accuracy and was agreed by all attending neonatologists and the investigator. The amount of blood for blood culture was calculated to make sure that at least one millilitre of blood volume was obtained, and this procedure was performed and checked by two staff nurses. Case report forms were verified for accuracy against source documents.

2.3.8 Data collection

Patient data were collected on individual case report forms (Appendix 9.7). These data included demographic data (gender, gestational age, and birth weight), maternal factors (modes of delivery, asphyxia, chorioamnionitis, and rupture of membranes), clinical characteristics (vital signs, cardiovascular and respiratory features, gastrointestinal signs and symptoms, neurological presentation, and skin lesions), laboratory results (complete blood count, C-reactive protein, blood glucose, serum lactate, total bilirubin, creatinine, alanine aminotransferase, electrolytes, blood gases, and coagulation test), diagnoses (considerations of sepsis, infectious conditions, comorbidities), treatments (antimicrobial

therapy, duration of mechanical ventilation, parenteral nutrition, central catheters, invasive intervention, shock management, blood products transfusion, and supportive care), and outcomes (mortality, length of stay, score of severity, severe sepsis, and septic shock). Disease severity was measured using the Neonatal Therapeutic Intervention Scoring System (NTISS) (Appendix 9.8) (113). I routinely recorded and collected demographic, clinical and laboratory information from the patients.

2.3.9 Data analysis

I performed univariable analysis by using Wilcoxon rank-sum test for continuous variables, and Chi-squared test or Fisher's exact test for categorical variables. All statistical analysis was performed using Stata version 14 and R version 3.4.2. A threshold of $p \leq 0.05$ was considered to be statistically significant. In the multivariable analysis for mortality outcome, Odds Ratio (OR), 95% Confidence Interval (95% CI), and *p*-values were estimated.

Empirical evidence and simulations show that to avoid overfitting (ideally) the number of predictors should not be larger than the number of deaths divided by 10. There were sixty-nine deaths in this study and thus, I enrolled seven variables into a model and used logistic regression analysis to find factors associated with mortality. The selection of these 7 variables was based on background knowledge and the clinical relevance. The lasso (least absolute shrinkage and selection operator) was considered a suitable approach to facilitate prediction and model selection for our setting.

2.4 Methods for the microbiology study

In chapter 5, I prospectively investigated the aetiology, microbial population structure, antimicrobial susceptibility patterns of the bacteria isolated from the blood culture of neonates with sepsis at CH1 in HCMC in Vietnam. The main aims of this study were 1) to identify the major bacterial causes of neonatal sepsis in our setting; 2) to investigate the susceptibilities of isolated bacteria to common antimicrobials such as cephalosporins, fluoroquinolones, carbapenems, oxacillin, and vancomycin; and 3) to evaluate potential associations between bacterial identification, the antimicrobial susceptibility patterns and mortality. The Strengthening the Reporting of Observational Studies in Epidemiology for New-born Infection (STROBE-NI) recommendations were used as standards for reporting in this study (142).

2.4.1 Bacterial culture and identification

One to three millilitres of blood were drawn aseptically before starting antimicrobial therapy and directly injected in Brain Heart Infusion (BHI) broth. The blood culture bottles were immediately incubated in a BACTEC blood culture system (Becton, Dickinson) at 37^{o}C for up to 5 days and sub-cultured on MacConkey's agar, sheep blood agar and chocolate agar. The inoculated MacConkey agar plates were incubated aerobically, whereas blood agar and chocolate agar plates were incubated in CO_2 incubator at 37^{o}C for 24–48 hours. Blood culture bottles showing no growth on sub-culture after 7 days of incubation were reported as negative. The identification of pathogens was performed using local protocols incorporating mass-spectrometry identification approaches with matrix-assisted laser desorption ionization – time of flight method (MALDI-TOF) (The Bruker MALDI Biotyper CA system, Bremen, Germany).

The pathogen-contaminant decision was made based on the clinical relevance of the isolated bacteria and the independent assessments by two qualified medical microbiologists. If there was disagreement, then the case was discussed until a consensus decision was reached.

2.4.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the pathogens isolated was performed by disk diffusion (Oxoid Thermo Fisher Scientific Ltd, Basingstoke, Hampshire, UK) using guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (145) the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (146) and, when required, by minimum inhibitory concentration estimation (MIC) using E test (bioMérieux, Craponne, France).

Antimicrobial susceptibility of isolated pathogens to selected antimicrobial agents were determined using the Kirby Bauer disc diffusion method. Pure colonies of isolates were emulsified to obtain 0.5 MacFarland standard and incubated on Miller Hinton agar. Antimicrobial discs were placed on the inoculated agar and incubated for 24 hours at 37^{o}C, we were then observed for zones of inhibition, breakpoints identified and determined as susceptible or resistant.

Antimicrobial susceptibilities for *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., *Enterobacter* spp. were determined against gentamicin (10 µg), amikacin (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (25 µg), amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), ticarcillin-clavulanic acid (85 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), nalidixic acid (30 µg), pefloxacin (5 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), and colistin (5 µg) (Oxoid Thermo Fisher Scientific Ltd, Basingstoke, Hampshire, UK).

Antimicrobial susceptibilities for CoNS and *Staphylococcus aureus* were determined against erythromycin (15 µg), pefloxacin (5 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), penicillin (10 µg), oxacillin (1 µg), cefoxitin (30 µg), clindamycin (2 µg), trimethoprim-sulfamethoxazole (25 µg), tetracycline (30 µg), rifampicin (30 µg), linezolid (30 µg), and vancomycin (30 µg) (Oxoid Thermo Fisher Scientific Ltd, Basingstoke, Hampshire, UK).

Antimicrobial susceptibilities for *Streptococcus* spp. were determined against erythromycin (15 µg), levofloxacin (5 µg), penicillin (10 µg), clindamycin (2 µg), tetracycline (30 µg), linezolid (30 µg), and vancomycin (30 µg) using standard disk diffusion method (Oxoid Thermo Fisher Scientific Ltd, Basingstoke, Hampshire, UK).

Escherichia coli ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used for antimicrobial susceptibility testing quality control.

2.4.3 Bacterial storage

Organisms were sub-cultured onto 5% blood agar and the purity of the isolate was tested before storage in BHI (Brain Heart Infusion) broth and 20% glycerol at –80°C.

2.4.4 Data analysis

Data are presented in numbers and percentages for categorical variables, median and interquartile range for continuous variables. Chi-squared test and Fisher's exact test was used for univariable analysis of categorical variables and Wilcoxon rank-sum test for continuous variables. Logistic regression was used for multivariable analysis to identify factors associated with mortality. All statistical analysis was performed using Stata version 14 and R version 3.4.2; $p \leq 0.05$ was considered to be significant.

2.5 Methods for the genomics study

2.5.1 Extraction of nucleic acids

DNA were extracted from bacterial isolates using the Wizard Genomic DNA Extraction Kit (Promega, Fitchburg, Wisconsin, USA). The quality and concentration of the DNA were assessed using a nano-drop spectrophotometer, and then Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) was used for DNA sequencing.

2.5.2 Genome sequencing

Whole genome sequencing was performed on DNA extracted from 15 *Acinetobacter baumannii* isolates collected in this study. One nanogram of DNA of each sample was used for preparing sequencing library according to the manufacturer's guidelines using a Nextera XT DNA Sample Preparation Kit (Illumina, Inc., San Diego, CA, USA) (147). Briefly, DNAs was fragmented using transposase and purified using AMPure XP Beads (Beckman Coulter, USA). The concentration of DNA libraries were determined using KAPPA Library Quantification Kit Illumina platforms (Kappa Biosystems, USA) (148). The DNA libraries were pooled in equimolar and run paired-end sequencing on an Illumina Miseq System. Raw reads generated were then assessed quality using FastQC (149). The passed reads were used for mapping and assembling. All FASTQ files for this study have been submitted to the European Nucleotide Archive (ENA) (https://www.ebi.ac.uk/ena).

2.5.3 Sequencing analysis

The RedDog pipeline V1beta.10.3 was used for mapping and calling all variants as previously described (150). Briefly, whole-genome sequences that had been passed

quality checking were first mapped to reference genome of *Acinetobacter baumannii* A1656-2 (Accession number: CP001921.1), using Bowtie 2 v2.2.9 with sensitive local mapping (151). All singe nucleotide polymorphisms (SNPs) with Phred score of ≥30 were then called by SAMTools v1.5.6 (152). Core SNPs that were defined with a minimum read depth of 5, mapping coverage ≥50%, and 90% conservations were concatenated to create an alignment of 3,948 variable sites among 15 isolates of *Acinetobacter baumannii*.

2.5.4 Phylogenetic trees

A maximum likelihood (ML) phylogenetic tree was constructed based on 3,948 core SNPs identified from mapping, using Randomized Accelerated Maximum Likelihood (RAxML) (153). RAxML v8.2.10 was run five times for the concatenation of these core SNPs, with the generalized time-reversible model with Γ distribution (GTR+ Γ), one hundred bootstrap pseudo-replicate analyses performing to assess the ML tree. The best phylogeny was selected as the one with the highest maximum likelihood values. The mid-point rooted phylogeny was used to plot gene contents in Interactive Tree Of Life (iTOL) (154).

2.5.5 Multilocus sequence typing and gene contents

SRST2 version 0.2.0 was run to map directly the reads to databases with default parameters for screening known alleles of multilocus sequence typing (MLST) and acquired antimicrobial resistance (AMR) genes (155). I used reference databases of MLST allele genes and resistance genes from the *Acinetobacter baumannii* BIGSdb database at Institute Pasteur (http://bigsdb.web.pasteur.fr) and the ARG-Annot database. Read sets were further assembled *de novo* by performing Unicycler to generate the contigs that then used to determine the capsule types.

2.5.6 Bayesian phylogenetic analysis

To infer the time scale where the outbreak occurred in CH1, the phylogeny of 25 isolates of ST570 was built using RAxML. Firstly, a pseudo-genome alignment of 25 isolates of ST570 (removed 3 duplicated isolates from same patients) was generated by matching SNPs to reference genome. Gubbins v2.2.0 was then used to scan the whole genome alignment to detect and remove potential recombination regions that could affect to further analysis (156). Gubbins left 131 SNPs among these 25 isolates. Checking by a phage search tool PHASTER, I detected 10 out of 131 SNPs were in bacterial phage regions (157). The concatenate of 121 non-recombination SNPs of 25 *Acinetobacter baumannii* ST570 isolates was finally used for BEAST analysis.

The TempEst v1.5 was used to test the best fit of linear regression between the age of samples and their root-to-tip distance (158). Further analysis to test temporal signal, the TipDatingBeast package in R was applied. This randomly reassigned the sampling dates of sequences for 20 times to create date-randomized data sets. Run BEAST analysis for these date-randomized sets and compared the mean rates between runs. The data was sufficient temporal signal when the mean rate of the correct sampling times was not contained in 95% credible intervals of mean rates of the date-randomized data set (159,160).

For selecting the best-fit model for BEAST analysis, I ran IQ-TREE on the free-recombination SNP alignment (121 variable sites), which was using the ultrafast and automatic model selection (ModelFinder) and was much faster than jModelTest and ProtTest (161). The ModelFinder showed that SYM+AIC was the lowest Bayesian

Information Criteria (BIC) and chosen as the best-fit substitution model. For BEAST analysis, I performed multiple runs on the data using the best-fit model, all equal base frequencies, constant size or Bayesian skyline demographic model, combined with a strict clock model, uncorrelated relaxed clock or random local clock. The path sampling and stepping-stone sampling approaches was applied to compare the log marginal likelihoods of these runs. The SYM+G with a strict clock and constant size was identified as the best-fit model. Finally, I ran BEAST three independent runs using the best-fit model, with the Markov Chain Monte Carlo (MCMC) of $10x10^6$ chains and sampled every 1000 iterations. Log files after three runs was combined using LogCombiner version 1.8.3, with a burn-in of 10%. Effective sample sizes (ESS) of all parameters, which were much higher than 500 was assessed by Tracer v1.6.0. The trees were combined and summarized using LogCombiner v1.8.3 and TreeAnnotator v1.8.3 (162).

3 The microbiology of bacterial bloodstream infections at Children's Hospital 1 in Ho Chi Minh City in Vietnam from 2013 to 2016

3.1 Introduction

BSIs contribute greatly to the morbidity and mortality of hospitalized children in LMICs (29,163,164). A positive blood culture remains the gold standard for the laboratory diagnosis of BSIs in children (165). In recent years, the management of BSIs has been threatened by dramatic increase of AMR. MDR Gram-positive and Gram-negative bacteria have resulted in difficult-to-treat or even untreatable BSIs (166). Paediatric AMR surveillance, that evaluates local epidemiological data is needed to improve clinical and laboratory evidence and ensure appropriate management of BSIs in children.

The World Health Organization (WHO) Global Antimicrobial Resistance Surveillance System (GLASS) (167) has listed six bacterial pathogens as the targets for regular AMR surveillance. These organisms are *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Salmonella* spp., *Staphylococcus aureus*, and *Streptococcus pneumoniae* (167). Measuring AMR in these pathogens is crucial for the treatment of BSIs in children in LMICs where empirical antimicrobials are in common use. A recent systematic review of AMR in children identified insufficient BSIs data from LMICs (168). In this review of 30 studies (7,056 positive blood cultures), Gram-negative bacteria accounted for 66.8% of all isolates (168). MDR in Gram-negative organisms was identified in 30% (interquartile range [IQR], 0–59.6) of organisms in Asia and 75% (IQR, 30–85.4) of organisms in Africa (168).

In Asia, BSIs in children caused by AMR pathogens are no longer a threat but an unwelcome reality (29,163,164). A retrospective study of BSIs in 588 children at a paediatric hospital in Siem Reap, Cambodia, from January 1, 2007, to July 31, 2011 (29) found an overall mortality of 19.0%. These cases were caused by a range of pathogenic bacteria, including *Staphylococcus aureus* (12.2%), *Streptococcus pneumoniae* (10.0%), *Klebsiella pneumoniae* (6.4%), and *Escherichia coli* (6.3%). AMR was particularly common among the Enterobacteriaceae (29). Researchers at the Children's Medical Centre Hospital in Tehran, Iran from January 2001 to December 2005 found that Gram-negative bacteria accounted for 52.4% of 2,581 positive blood cultures (163). The prevalence of MRSA and methicillin-resistant CoNS were 79% and 89%, respectively. Among the Gram-negative isolates, *Pseudomonas aeruginosa* was the most common bacteria (163). A further study from Nepal described two outbreaks of MDR *Klebsiella pneumoniae* in 48 children with a mortality rate of 75% (164).

Contemporary data regarding the microbiology of BSIs in children in LMICs are scarce (168). We know little about contemporary antimicrobial susceptibility patterns in the major bacterial causes of BSIs in children in Vietnam. I hypothesized that there has been a dramatic surge in MDR Gram-negative organisms and the emergence of MRSA. Therefore, to better understand these the distribution of organisms and to inform antimicrobial treatment regimens, I aimed to describe the bacterial aetiology of BSIs and to investigate the prevalence of AMR at CH1 in Vietnam from 2013 to 2016.

3.2 Results

3.2.1 Bacteria isolated from blood culture at CH1 between 2013 and 2016

The overall positivity of blood cultures at CH1 was 7.6% (1,050/13,727) in 2013, 8.1% (1,086/13,444) in 2014, 7.4% (1,180/15,888) in 2015, and 8.0% (1,379/17,294) in 2016. Over this study period (2013 to 2016) there was wide variation in the number of bacterial pathogens isolated from the blood of febrile children from all ages at CH1. Notably, there was an increasing number of bacteria isolated from blood culture, rising from 1,050 bacteria in 2013 to 1,379 bacteria in 2016. There was also a noticeable reduction in the proportion of bacteria causing BSIs displaying susceptibility to antimicrobials during this 4-year period.

The most common Gram-negative bacteria isolated from blood over the sampling period were *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. *Acinetobacter* spp. made up the highest proportion of Gram-negative bacterial profile from 2013 to 2016. The most common Gram-positive organisms were CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. The number of CoNS increased to comprise 809/1,379 (58.7%) of all isolates at the end of the 4-year period.

Other organisms isolated during this period were *Burkholderia* spp., *Enterobacter* spp., *Salmonella* spp., *Morganella morganii*, *Moraxella* spp., *Elizabethkingia meningoseptica*, *Haemophilus influenzae*, *Neisseria* spp., *Serratia* spp., *Enterococcus* spp., and *Streptococcus agalactiae*. The numbers of these organisms were all <30 isolates annually between 2013 and 2016. There were *Burkholderia* spp. (15 isolates in 2013, 12 isolates in 2014, 9 isolates in 2015, and 25 isolates in 2016); *Enterobacter* spp. (17, 20, 16, 9); *Salmonella* spp. (7, 11, 15, 8); *Morganella morganii* (16, 2, 2, 1); *Moraxella* spp. (0, 0, 5,

6); *Elizabethkingia meningoseptica* (0, 0, 9, 4); *Haemophilus influenzae* (4, 3, 2, 4); *Neisseria* spp. (4, 0, 0, 4); *Serratia* spp. (3, 3, 2, 3); *Enterococcus* spp. (18, 15, 20, 16); and *Streptococcus agalactiae* (GBS) (2, 4, 5, 6) throughout the 4-year period. No isolates of *Listeria monocytogenes* were isolated during the study period.

I found no significant difference in the proportional change of *Acinetobacter* spp. (p=0.271), *Escherichia coli* (p=0.810), *Klebsiella* spp. (p=0.839), *Pseudomonas* spp. (p=0.447), CoNS (p=0.494), *Staphylococcus aureus* (p=0.974), and *Streptococcus pneumoniae* (p=0.986) between 2013 and 2016 when comparing proportions using Chi-squared test. The proportion and trends of major bacteria isolated from the blood culture of all children at CH1 between 2013 and 2016 are shown in Table 3.1 and Figure 3.1.

Table 3.1 Major bacteria isolated from blood culture of children of all ages at CH1 between 2013 and 2016

Bacterial species	2013 N=1,050	2014 N=1,086	2015 N=1,180	2016 N=1,379	*P*-values*
Acinetobacter spp.	122 (11.6)	93 (8.6)	56 (4.7)	83 (6.0)	0.271
Escherichia coli	44 (4.2)	66 (6.1)	42 (3.6)	51 (3.7)	0.810
Klebsiella spp.	46 (4.4)	44 (4.1)	29 (2.5)	37 (2.7)	0.839
Pseudomonas spp.	73 (7.0)	43 (4.0)	38 (3.2)	38 (2.8)	0.447
CoNS	562 (53.5)	581 (53.5)	739 (62.6)	809 (58.7)	0.494
Staphylococcus aureus	42 (4.0)	43 (4.0)	54 (4.6)	46 (3.3)	0.974
Streptococcus pneumoniae	21 (2.0)	26 (2.4)	27 (2.3)	39 (2.8)	0.986

Values are n (%), *Chi-squared test.

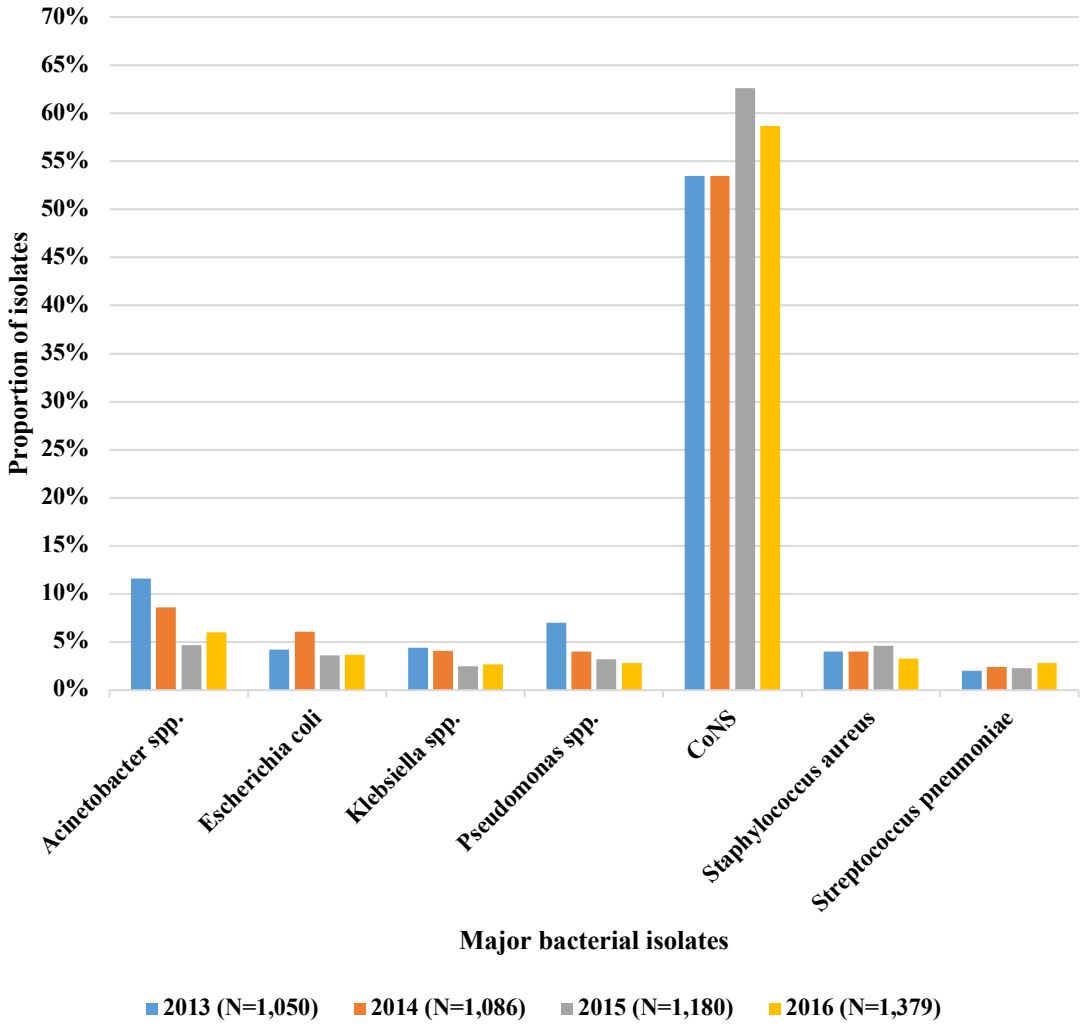

Figure 3.1 The proportion of major isolated bacteria from blood culture in children at CH1 over the study period between 2013 and 2016

The proportion of major bacteria including *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae* isolated from blood culture in children of all ages at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was no significant change in the trend of major bacterial isolates during the four years of study.

3.2.2 Bacteria isolated from blood culture in children ≤1 month of age

In neonates, *Acinetobacter* spp. accounted for the largest number of Gram-negative bacteria, followed by *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. between 2013 and 2016. Notably, *Pseudomonas* spp. saw a decline in numbers from 2013 to 2016. The number of *Staphylococcus aureus* increased marginally with time, but the number of CoNS rose sharply from 180 isolates in 2013 to 315 in 2016. Additionally, *Streptococcus pneumoniae* was determined not to be a significant pathogen in this neonatal population. Between 2013 and 2016, there was no significant change in the trend of major bacteria isolated from blood culture in children ≤1 month of age (Chi-squared test). Major bacteria isolated from blood culture in children ≤1 month of age between 2013 and 2016 are shown in Figure 3.2.

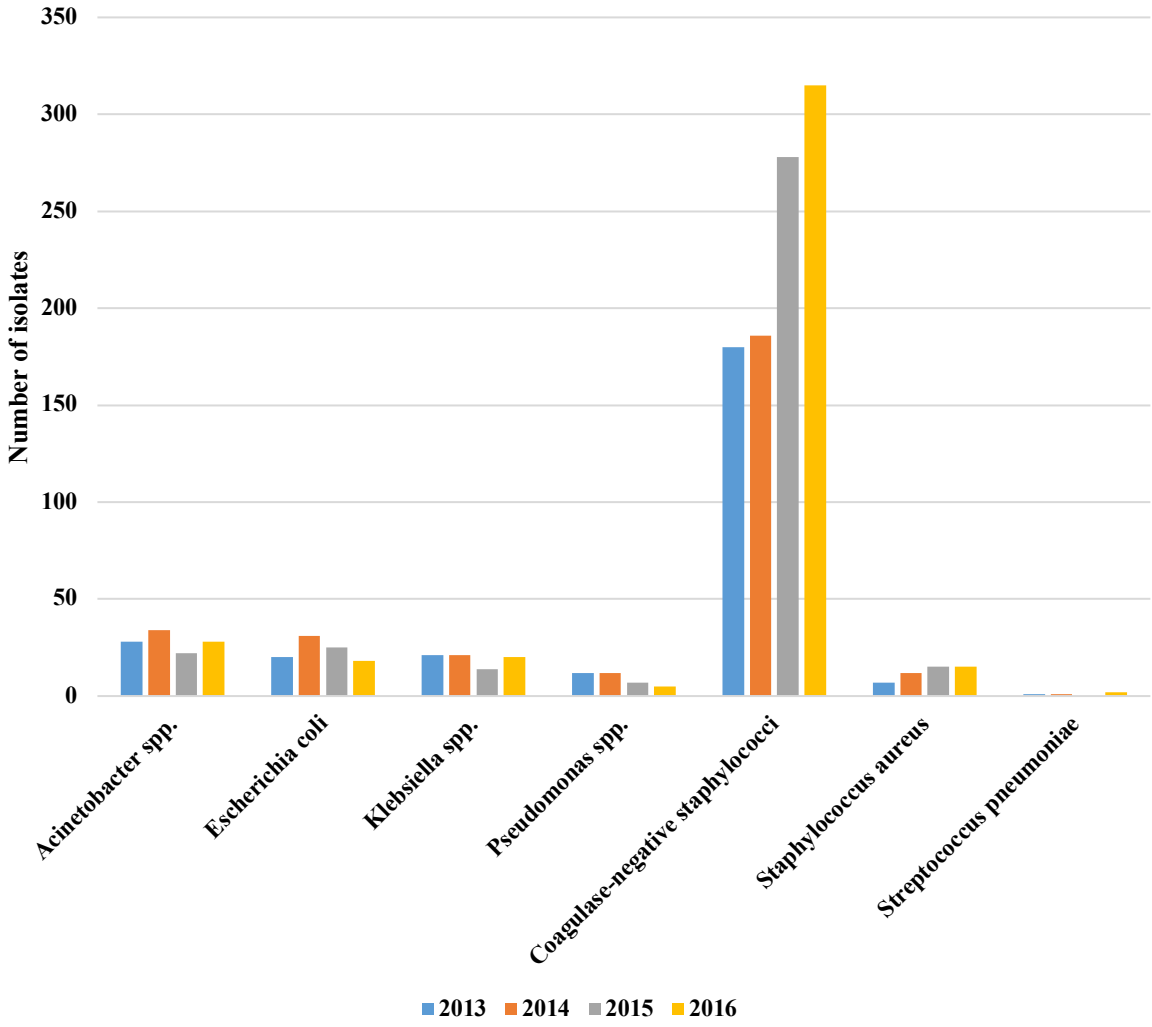

Figure 3.2 Bacteria isolated from blood culture in children ≤1 month of age

Major bacteria including *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae* isolated from blood culture in children ≤1 month of age (neonates) at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was no significant change in the trend of major isolates in neonatal population during the four years of study.

3.2.3 Bacteria isolated from blood culture in children >1 month–1 year of age

In children aged >1 month–1 year of age, *Acinetobacter* spp., *Escherichia coli*, and *Klebsiella* spp., remained the major Gram-negative pathogens; however, their numbers declined considerably in 2015 and then again increased in 2016. The number of *Pseudomonas* spp. declined gradually from 36 isolates in 2013 to 15 isolates in 2016. CoNS remained a significant Gram-positive bacterium. *Staphylococcus aureus* and *Streptococcus pneumoniae* constituted the majority of Gram-positive pathogens in this age range. Between 2013 and 2016, there was no significant change in the trend of major bacteria isolated from blood culture in children >1 month–1 year of age (Chi-squared test). Major bacteria isolated from blood culture in children >1 month–1 year of age between 2013 and 2016 are shown in Figure 3.3.

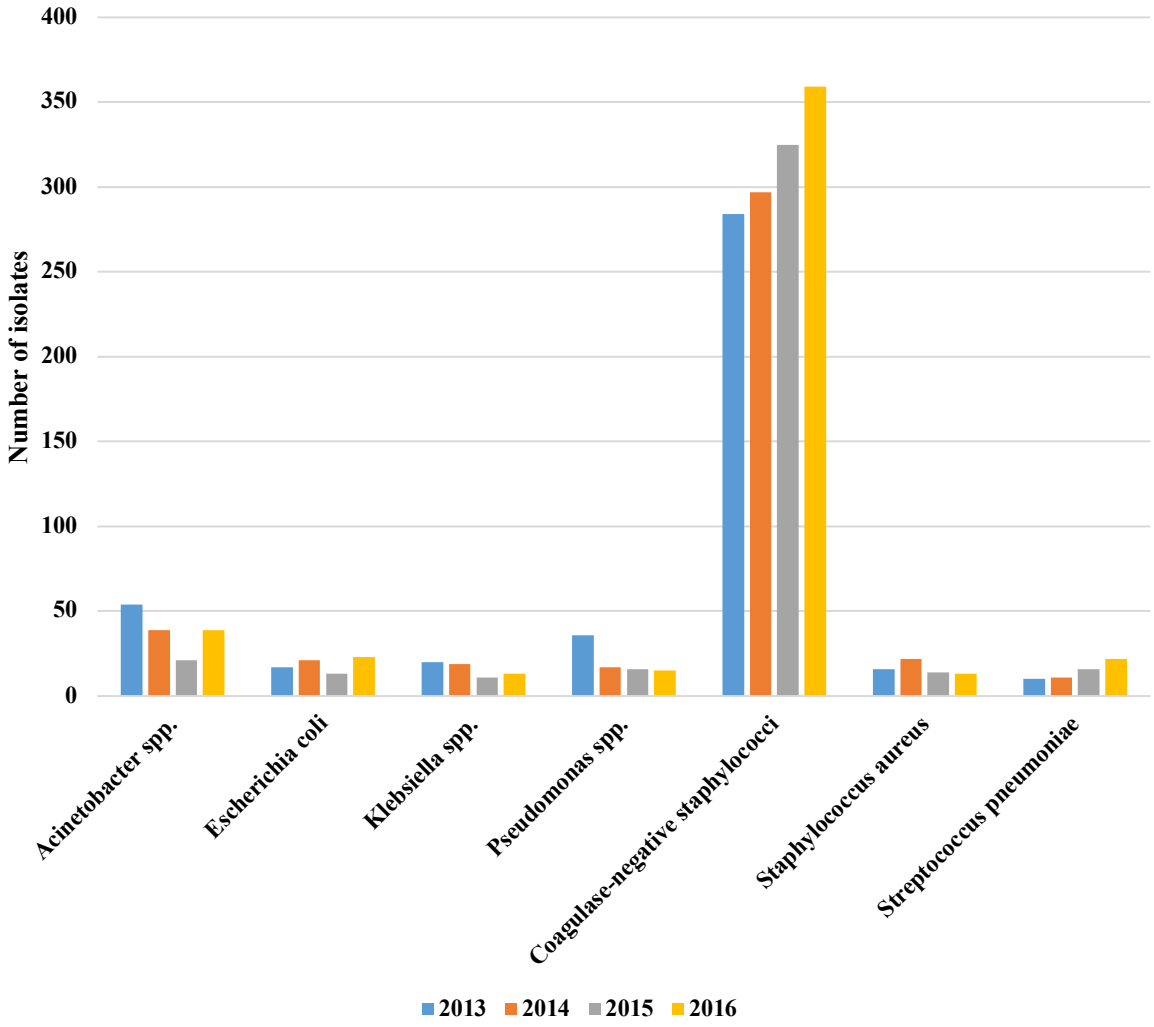

Figure 3.3 Bacteria isolated from blood culture in children >1 month–1 year of age

Major bacteria including *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae* isolated from blood culture in children >1 month–1 year of age at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was no significant change in the trend of major isolates in children >1 month–1 year of age during the four years of study.

3.2.4 Bacteria isolated from blood culture in children >1 year–6 years of age

In comparison to the number of bacterial isolates from neonates and infants, children aged >1 year–6 years contributed a notably lower number of bacterial isolates over the 4-year period. *Acinetobacter* spp. were still the most common Gram-negative pathogen; however, *Pseudomonas* spp. replaced *Escherichia coli* and *Klebsiella* spp. to be the second most common pathogen. CoNS, *Staphylococcus aureus*, *Streptococcus pneumoniae* constituted the vast majority of the Gram-positive bacteria. However, it was apparent that these bacteria were less common in children >1 year–6 years compared with that in neonates and infants. Between 2013 and 2016, there was no significant change in the trend of major bacteria isolated from blood culture in children >1 year–6 years of age (Chi-squared test). Major bacteria isolated from blood culture in children >1 year–6 years of age between 2013 and 2016 are shown in Figure 3.4.

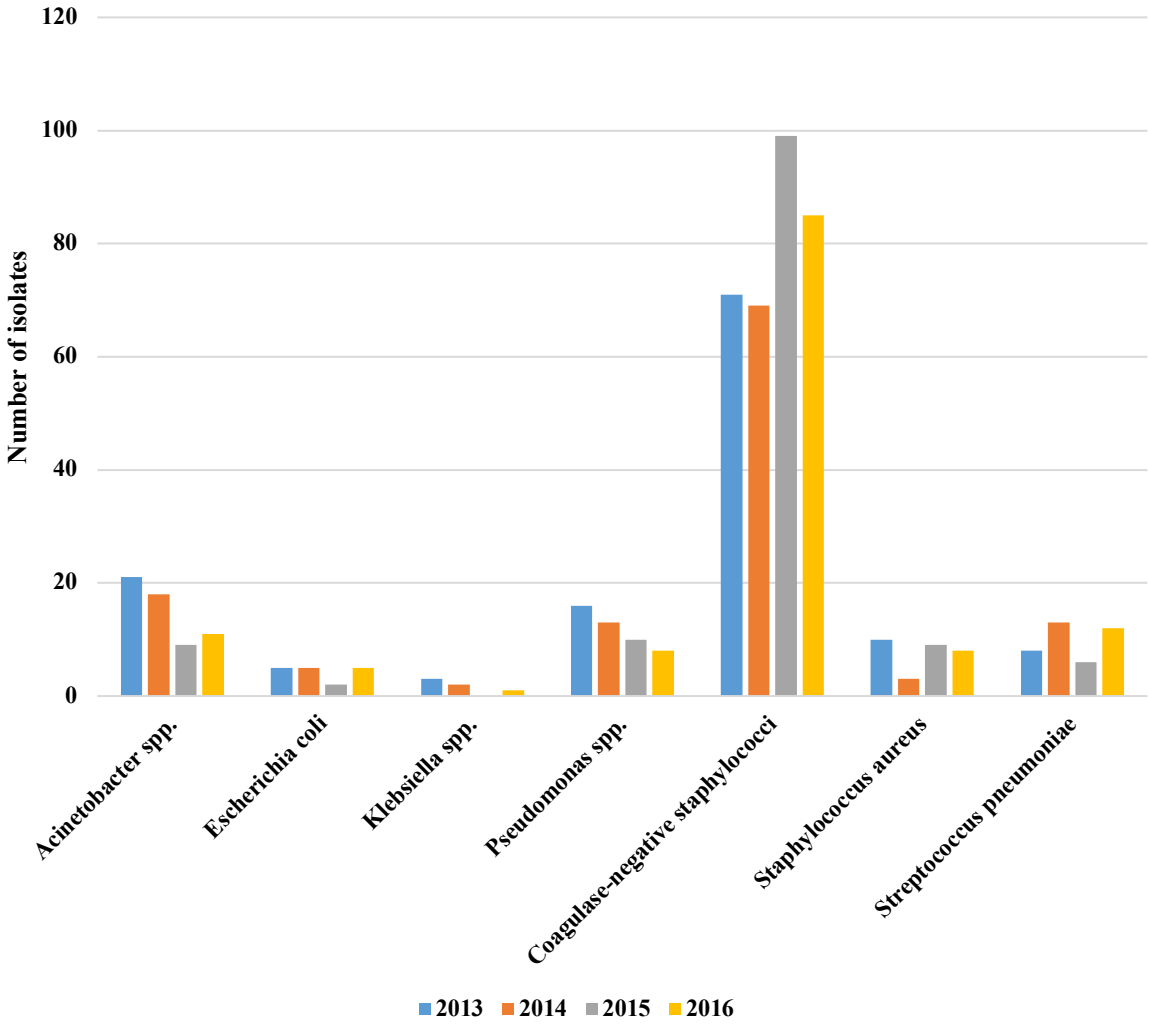

Figure 3.4 Bacteria isolated from blood culture in children >1 year–6 years of age

Major bacteria including *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae* isolated from blood culture in children >1 year–6 years of age at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was no significant change in the trend of major isolates in children >1 year–6 years of age during the four years of study.

3.2.5 Bacteria isolated from blood culture in children >6 years of age

In children >6 years of age, the number of *Klebsiella* spp. isolates remained between 2 to 4 cases per year between 2013 to 2016. During this 4-year period, *Acinetobacter* spp. contributed the highest number of isolates in 2013 with 19 cases, before it declined to 2 cases in 2014, before increasing to 5 isolates in 2016. A similar trend was observed with *Pseudomonas* spp. with a significant decrease in the number of isolates in 2014. Overall, *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. were the most common Gram-negative pathogens associated with BSIs in children >6 years of age. CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae* contributed considerably to Gram-positive bacterial profile, with CoNS showing an upward trend over the 4-year period. Between 2013 and 2016, I found no significant change in the trend of major bacteria isolated from blood culture in children >6 years of age (Chi-squared test). Major bacteria isolated from blood culture in >6 years of age between 2013 and 2016 are shown in Figure 3.5.

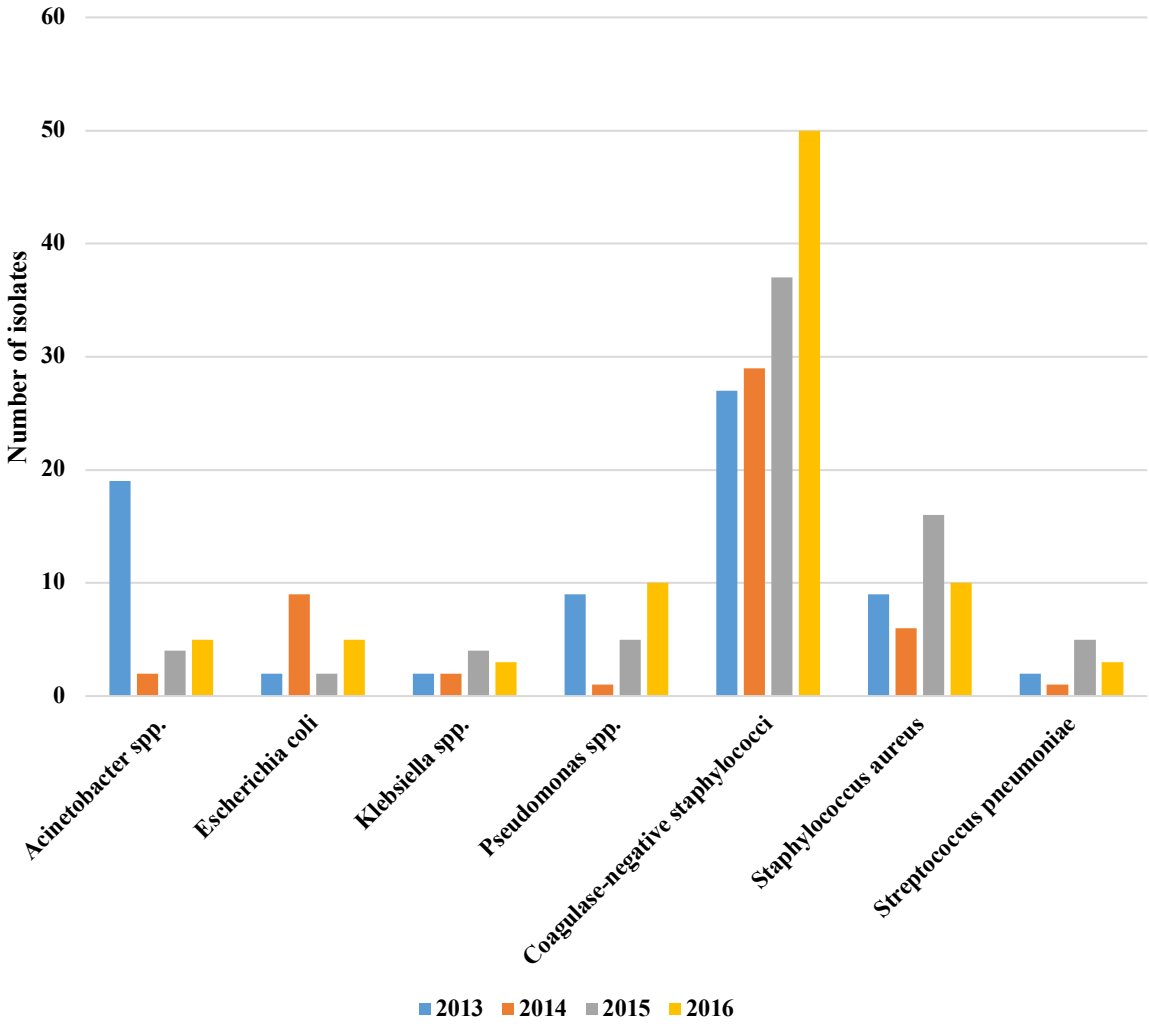

Figure 3.5 Bacteria isolated from blood culture in children >6 years of age

Major bacteria including *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae* isolated from blood culture in children >6 years of age at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was no significant change in the trend of major isolates in children >6 years of age during the four years of study.

3.2.6 The antimicrobial susceptibility of *Acinetobacter* spp. isolated from blood culture of children at CH1 between 2013 and 2016

Acinetobacter spp. were the most common genus of Gram-negative bacteria isolated from blood of children attending CH1 between 2013 and 2016. However, the overall proportion of *Acinetobacter* spp. decreased over the data collection period with 122/1,050 isolates (11.6%) in 2013; 93/1,086 isolates (8.6%) in 2014; 56/1,180 isolates (4.7%) in 2015; and 83/1,379 isolates (6.0%) in 2016.

The isolated *Acinetobacter* spp. demonstrated resistance to all classes of antimicrobials that were used from 2013 to 2016. Critically, for imipenem and meropenem, which are known as "last-resort" antimicrobials to treat Gram-negative bacteraemia, the susceptibility of *Acinetobacter* spp. in 2016 was 61.1% and 51.6%, respectively. The susceptibility of *Acinetobacter baumannii* to colistin was not tested during this 4-year period of study. However, I observed that *Acinetobacter* spp. had a high prevalence of susceptibility against trimethoprim/sulfamethoxazole (56.1% in 2016) (Figure 3.6). Notably, between 2013 and 2016, I found a significant increase in the susceptibility of *Acinetobacter* spp. to cefotaxime (20.5%, 31.4%, 31.4%, and 48.3%; respectively; $p<0.001$; Chi-squared test).

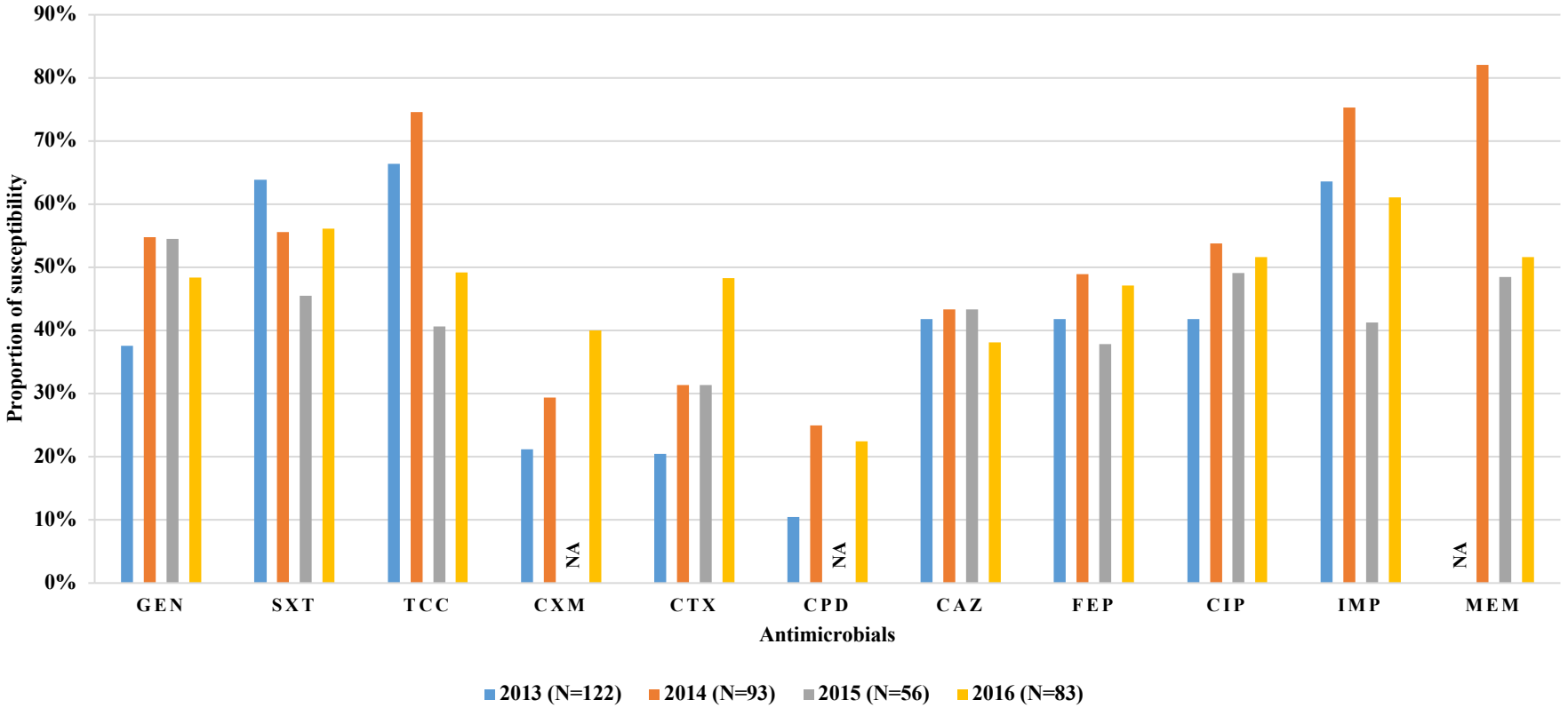

Figure 3.6 Antimicrobial susceptibility of *Acinetobacter* spp. isolated from blood culture of children at CH1 between 2013 and 2016
The antimicrobial susceptibility proportions of *Acinetobacter* spp. isolated from blood culture at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was a significant increase in the susceptibility of *Acinetobacter* spp. to cefotaxime between 2013 and 2016. GEN: gentamicin, SXT: trimethoprim-sulfamethoxazole, TCC: ticarcillin-clavulanic acid, CXM: cefuroxime, CTX: cefotaxime, CPD: cefpodoxime, CAZ: ceftazidime, FEP: cefepime, CIP: ciprofloxacin, IMP: imipenem, MEM: meropenem, NA: not available.

3.2.7 Antimicrobial susceptibility of *Escherichia coli* isolated from blood culture of children at CH1 between 2013 and 2016

There was high level resistance in *Escherichia coli* to all tested antimicrobials including cephalosporins (susceptibility <50% for cefotaxime, cefpodoxime, ceftazidime, cefepime) and fluoroquinolones (susceptibility raging from 20.7% to 53.8% for pefloxacin and from 35.4% to 50.0% for ciprofloxacin). Notably, the empirical antimicrobials that are commonly selected at CH1 (ampicillin, cefotaxime, and gentamicin) may not be effective as once predicted; *Escherichia coli* common had reduced susceptibility to ampicillin (only 6.0% in 2016), cefotaxime (39.3% in 2014), and gentamicin (49.4% in 2016). Fortunately, the susceptibilities of *Escherichia coli* to imipenem (88.0%), meropenem (90.4%), and ertapenem (87.7%) in 2016 remained high (Figure 3.7). Between 2013 and 2016, I identified a significant reduction in susceptibility of *Escherichia coli* to ampicillin (25.0%, 12.3%, 8.8%, and 6.0%; respectively; $p<0.001$; Chi-squared test) and to ceftazidime (47.7%, 44.2%, 25.9%, and 4.1%; respectively; $p<0.001$; Chi-squared test).

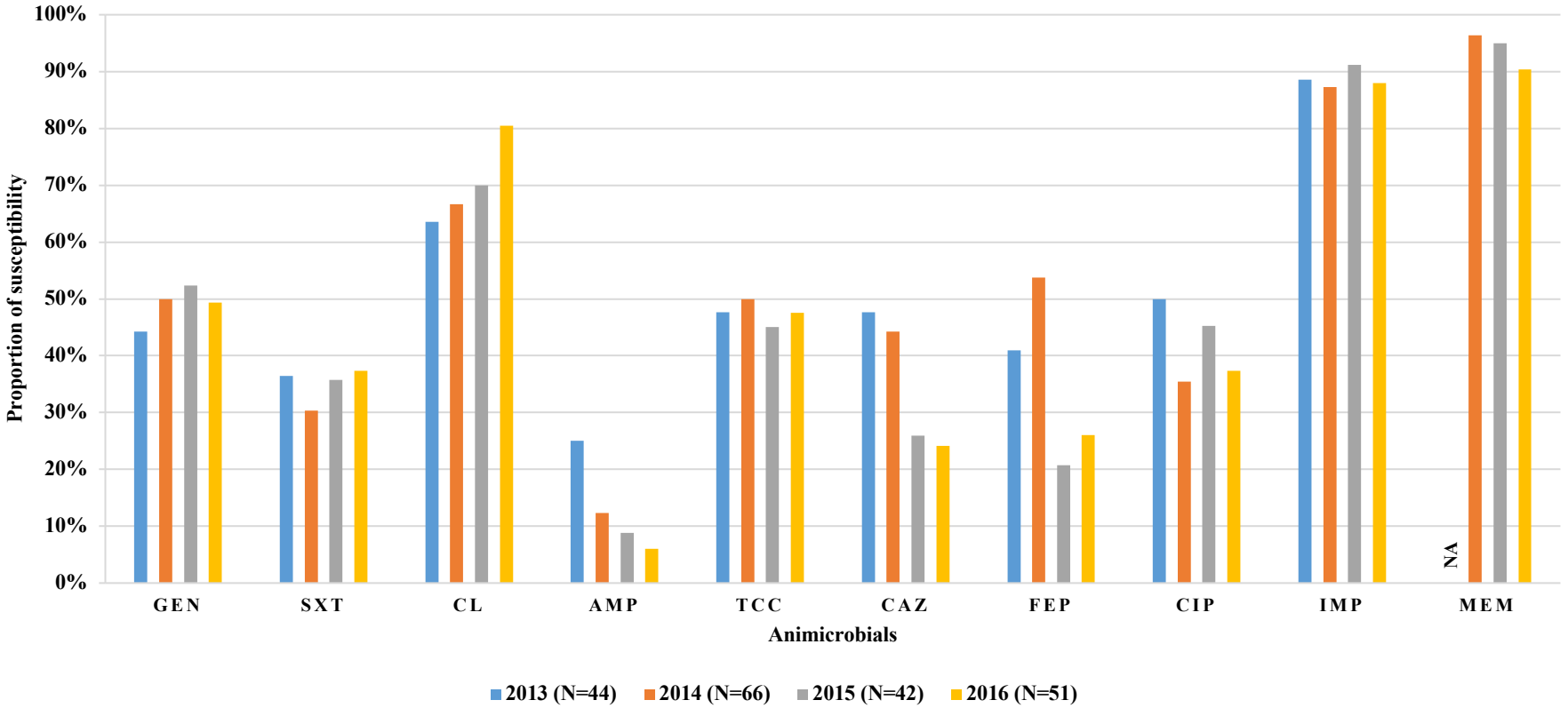

Figure 3.7 Antimicrobial susceptibility of *Escherichia coli* isolated from blood culture of children at CH1 between 2013 and 2016
The antimicrobial susceptibility proportions of *Escherichia coli* isolated from blood culture at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was a significant reduction in the susceptibility of *Escherichia coli* to ampicillin and to ceftazidime between 2013 and 2016. GEN: gentamicin, SXT: trimethoprim-sulfamethoxazole, CL: chloramphenicol, AMP: ampicillin, TCC: ticarcillin-clavulanic acid, CAZ: ceftazidime, FEP: cefepime, CIP: ciprofloxacin, IMP: imipenem, MEM: meropenem, ETP: ertapenem, NA: not available.

3.2.8 Antimicrobial susceptibility of *Klebsiella* spp. isolated from blood culture of children at CH1 between 2013 and 2016

During the 4-year period from 2013 to 2016, *Klebsiella* spp. showed a significant escalation in resistance against many antimicrobials. This trend could be observed in 2016 when the susceptibility was ominously low against some key antimicrobials, such as ticarcillin/clavulanic acid (19.6%), ceftazidime (7.7%), cefepime (23.5%), and ciprofloxacin (17.3%). The susceptibility of *Klebsiella* spp. against the carbapenems was also alarmingly also low and was 57.7% for imipenem, 50.0% for meropenem, and 57.7% for ertapenem in 2016 (Figure 3.8). There was no significant change in the trend of antimicrobial susceptibilities for *Klebsiella* spp. between 2013 and 2016 (Chi-squared test).

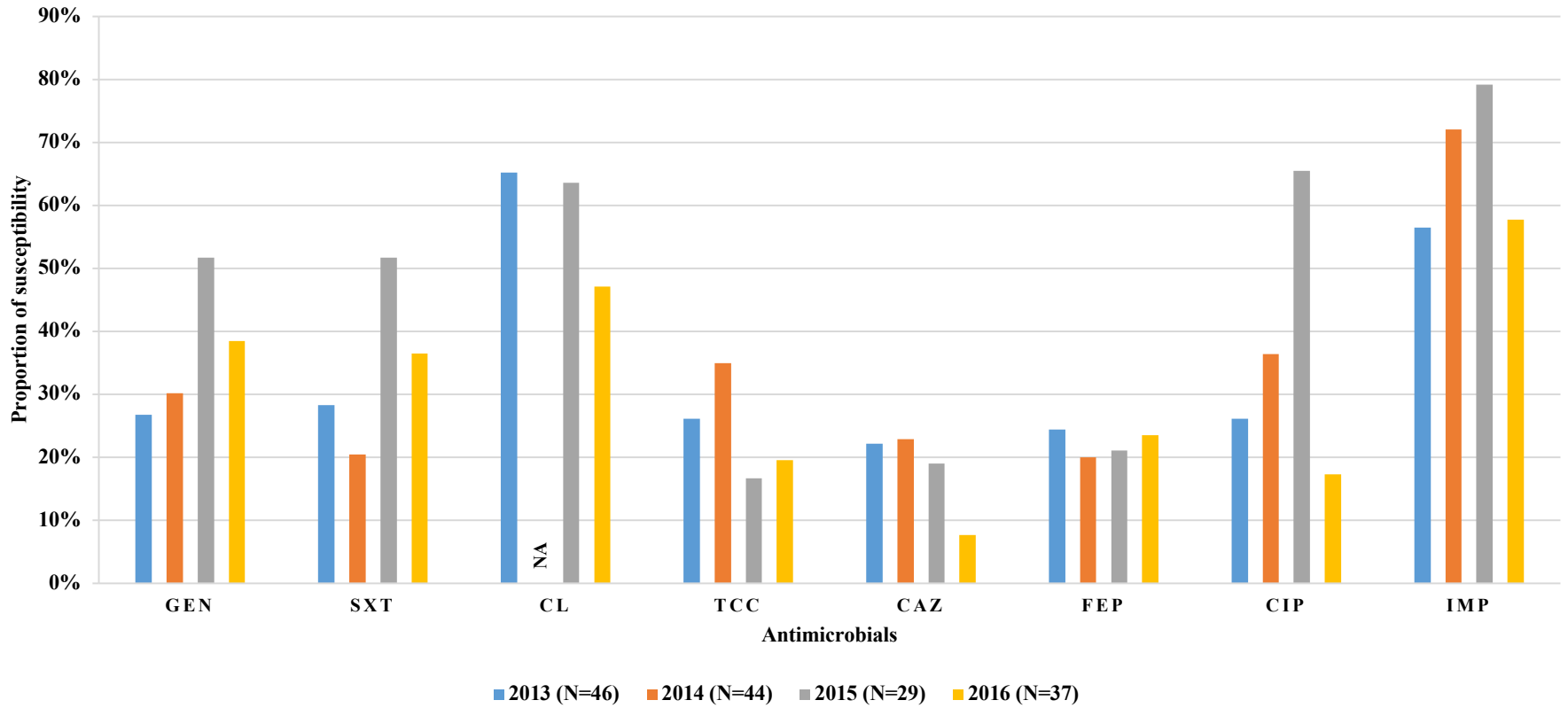

Figure 3.8 Antimicrobial susceptibility of *Klebsiella* spp. isolated from blood culture of children at CH1 between 2013 and 2016
The antimicrobial susceptibility proportions of *Klebsiella* spp. isolated from blood culture at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was no significant change in the trend of antimicrobial susceptibilities for *Klebsiella* spp. between 2013 and 2016. GEN: gentamicin, SXT: trimethoprim-sulfamethoxazole, CL: chloramphenicol, TCC: ticarcillin-clavulanic acid, CAZ: ceftazidime, FEP: cefepime, CIP: ciprofloxacin, IMP: imipenem, NA: not available.

3.2.9 Antimicrobial susceptibility of *Pseudomonas* spp. isolated from blood culture of children at CH1 between 2013 and 2016

Over the period of investigation, I observed some variation in the proportion of *Pseudomonas* spp. that were susceptible to commonly used antimicrobials. These 4-year trends were generally associated with a decrease in resistance. For example, susceptibility to ceftazidime increased from 46.6% in 2013 to 74.6% in 2016, a similar pattern was observed for cefepime (49.1% in 2013 to 75.5% in 2016) and for ciprofloxacin (41.4% in 2013 to 75.9% in 2016). Most notably susceptibility to imipenem increased from 70.7% in 2013 to 100% in 2016 (Figure 3.9). I found no significant change in the trend of antimicrobial susceptibilities for *Pseudomonas* spp. between 2013 and 2016 (Chi-squared test).

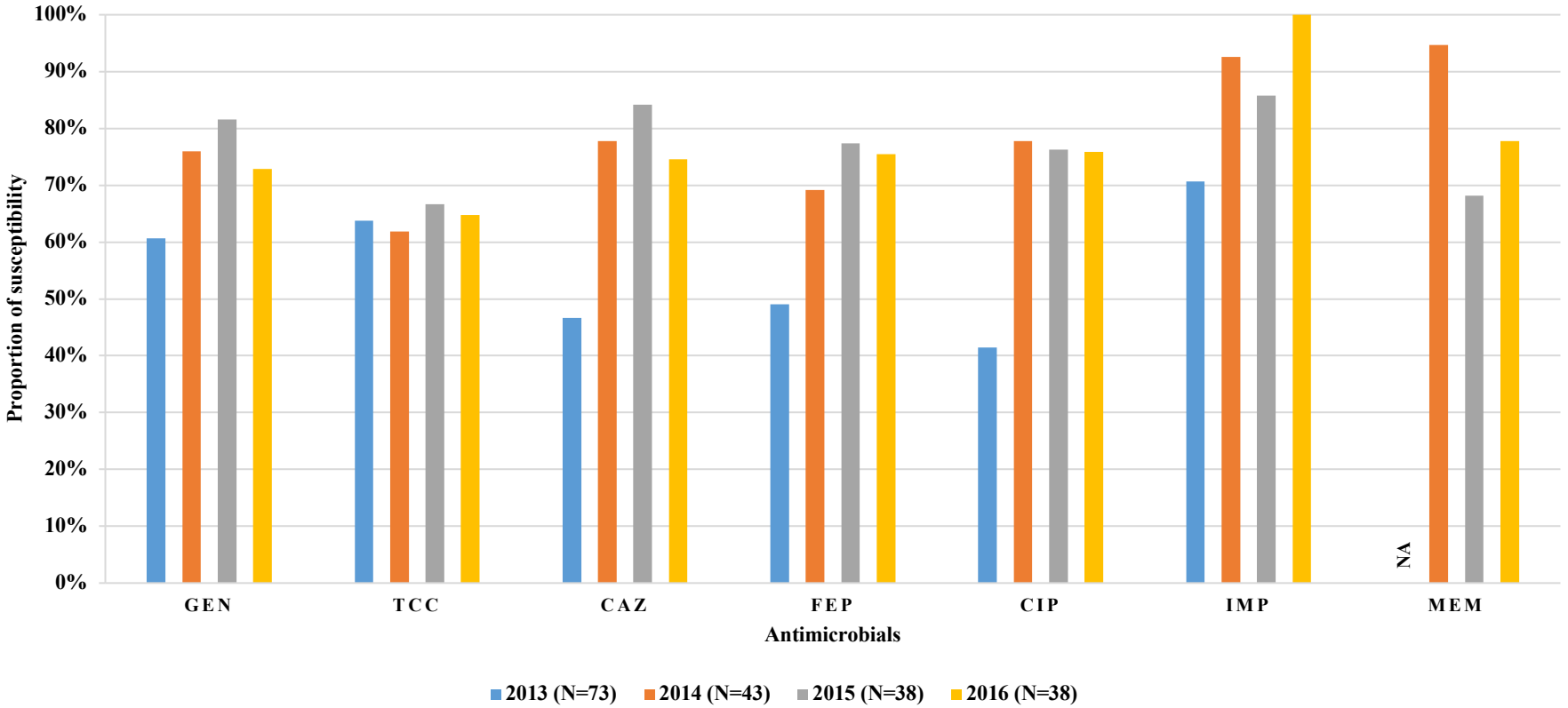

Figure 3.9 Antimicrobial susceptibility of *Pseudomonas* spp. isolated from blood culture of children at CH1 between 2013 and 2016
The antimicrobial susceptibility proportions of *Pseudomonas* spp. isolated from blood culture at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was no significant change in the trend of antimicrobial susceptibilities for *Pseudomonas* spp. between 2013 and 2016. GEN: gentamicin, TCC: ticarcillin-clavulanic acid, CAZ: ceftazidime, FEP: cefepime, CIP: ciprofloxacin, IMP: imipenem, MEM: meropenem, NA: not available.

3.2.10 Antimicrobial susceptibility of CoNS isolated from blood culture of children at CH1 between 2013 and 2016

From 2013 to 2016, of CoNS to oxacillin was consistently <10%, with a slight increase in susceptibility over the 4-year period from 2.1% in 2013 to 9.2% in 2016. The susceptibility of CoNS to vancomycin was 99.5% in 2015 and 99.8% in 2016 (Figure 3.10). I identified a significant increase in the susceptibility of CoNS to oxacillin between 2013 and 2016 (2.1%, 2.6%, 3.0%, and 9.2%; respectively; p=0.041; Chi-squared test), although the proportions were all <10%.

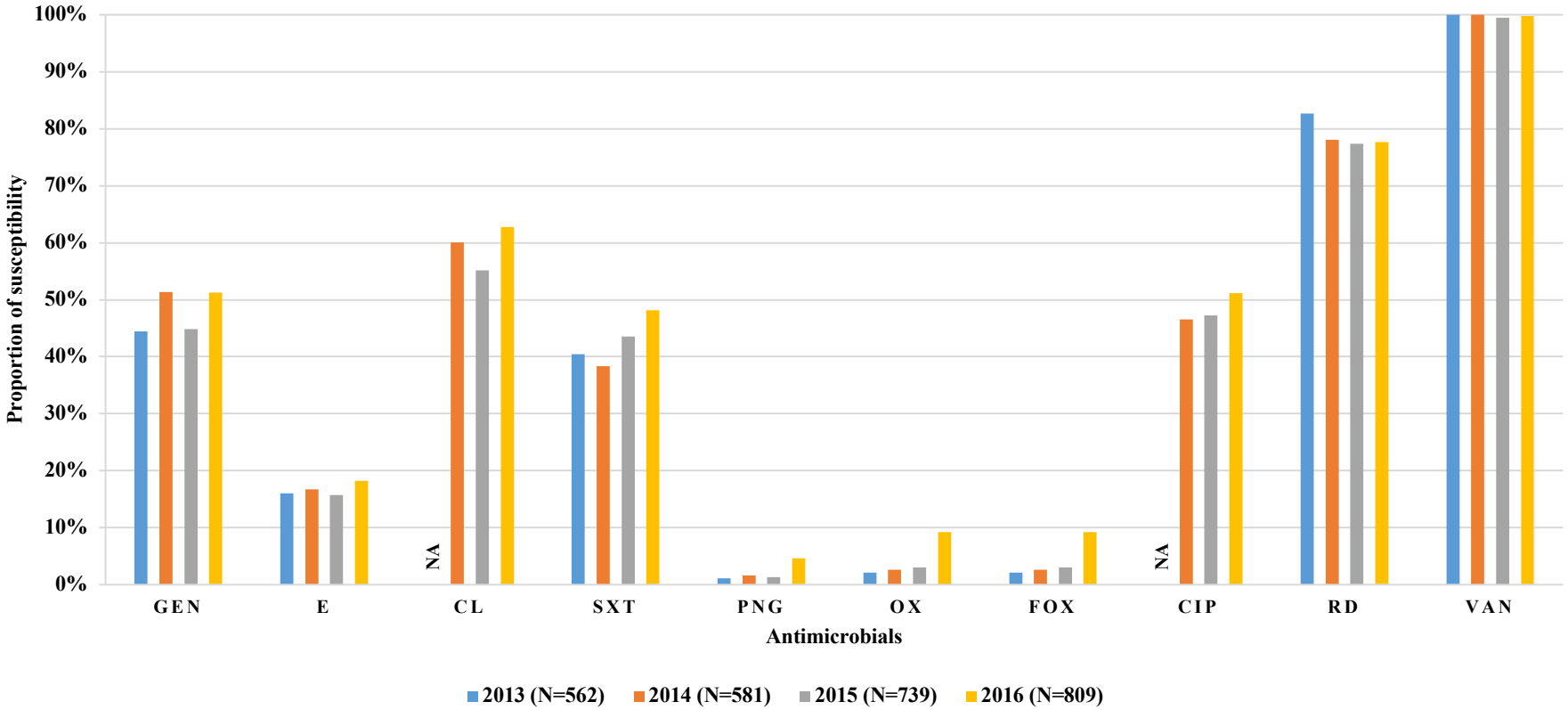

Figure 3.10 Antimicrobial susceptibility of CoNS isolated from blood culture of children at CH1 between 2013 and 2016

The antimicrobial susceptibility proportions of CoNS isolated from blood culture at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was a significant increase in the susceptibility of CoNS to oxacillin between 2013 and 2016. GEN: gentamicin, E: erythromycin, CL: chloramphenicol, SXT: trimethoprim-sulfamethoxazole, PNG: penicillin G, OX: oxacillin, FOX: cefoxitin, CIP: ciprofloxacin, RD: rifampicin, VAN: vancomycin, NA: not available.

3.2.11 Antimicrobial susceptibility of *Staphylococcus aureus* isolated from blood culture of children at CH1 between 2013 and 2016

Overall, the susceptibility of *Staphylococcus aureus* to oxacillin decreased from 23.8% in 2013 to 16.7% in 2016; 2014 saw a significant decline to 11.6%. However, the susceptibility of *Staphylococcus aureus* to vancomycin showed no change, remaining constant at 100% during a period of 4 years from 2013 to 2016 (Figure 3.11). I identified a significant increase in the susceptibility of *Staphylococcus aureus* to ciprofloxacin from 2013 to 2016 (36.6%, 63.8%, and 69.7%; respectively; $p<0.001$; Chi-squared test).

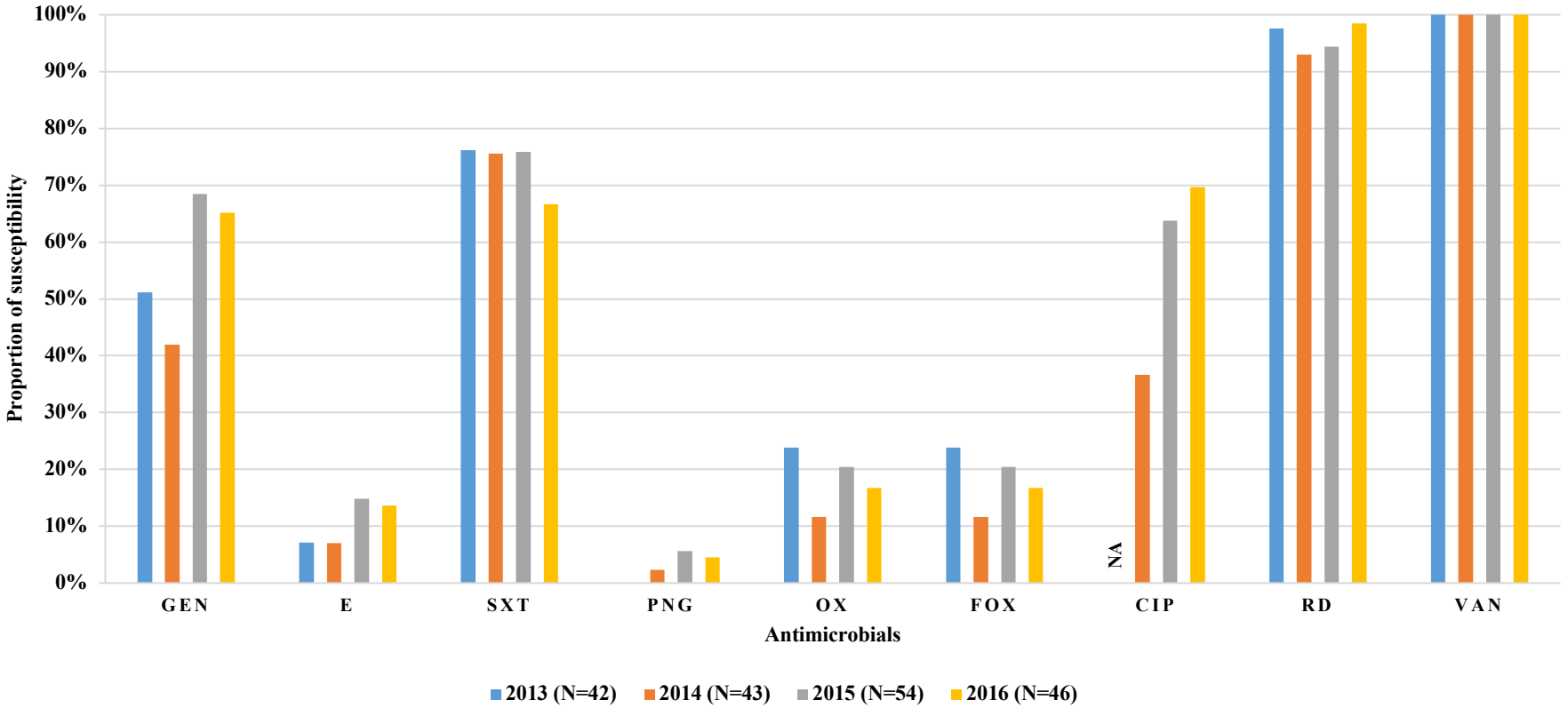

Figure 3.11 Antimicrobial susceptibility of *Staphylococcus aureus* isolated from blood culture of children between 2013 and 2016
The antimicrobial susceptibility proportions of *Staphylococcus aureus* isolated from blood culture at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was a significant increase in the susceptibility of *Staphylococcus aureus* to ciprofloxacin from 2013 to 2016. GEN: gentamicin, E: erythromycin, SXT: trimethoprim-sulfamethoxazole, PNG: penicillin G, OX: oxacillin, FOX: cefoxitin, CIP: ciprofloxacin, RD: rifampicin, VAN: vancomycin, NA: not available.

3.2.12 Antimicrobial susceptibility of *Streptococcus pneumoniae* isolated from blood culture of children at CH1 between 2013 and 2016

Susceptibility to penicillin G in *Streptococcus pneumoniae* decreased from 85.7% in 2013 to 60.7% in 2016. Alternatively, the proportion of *Streptococcus pneumoniae* with susceptibility against trimethoprim/sulfamethoxazole increased from 0.0% in 2013 to 12.1% in 2016. Overall, 23.8% of all *Streptococcus pneumoniae* were susceptible to ciprofloxacin in 2014 and 96.7% of organisms were susceptible levofloxacin in 2016. The proportion of *Streptococcus pneumoniae* that were susceptible to vancomycin remained at 100% during the 4-year period (Figure 3.12). I found a significant decrease in the susceptibility of *Streptococcus pneumoniae* against penicillin G from 2013 to 2016 (85.7%, 83.3%, 80.0%, and 60.7%; respectively; $p<0.001$; Chi-squared test).

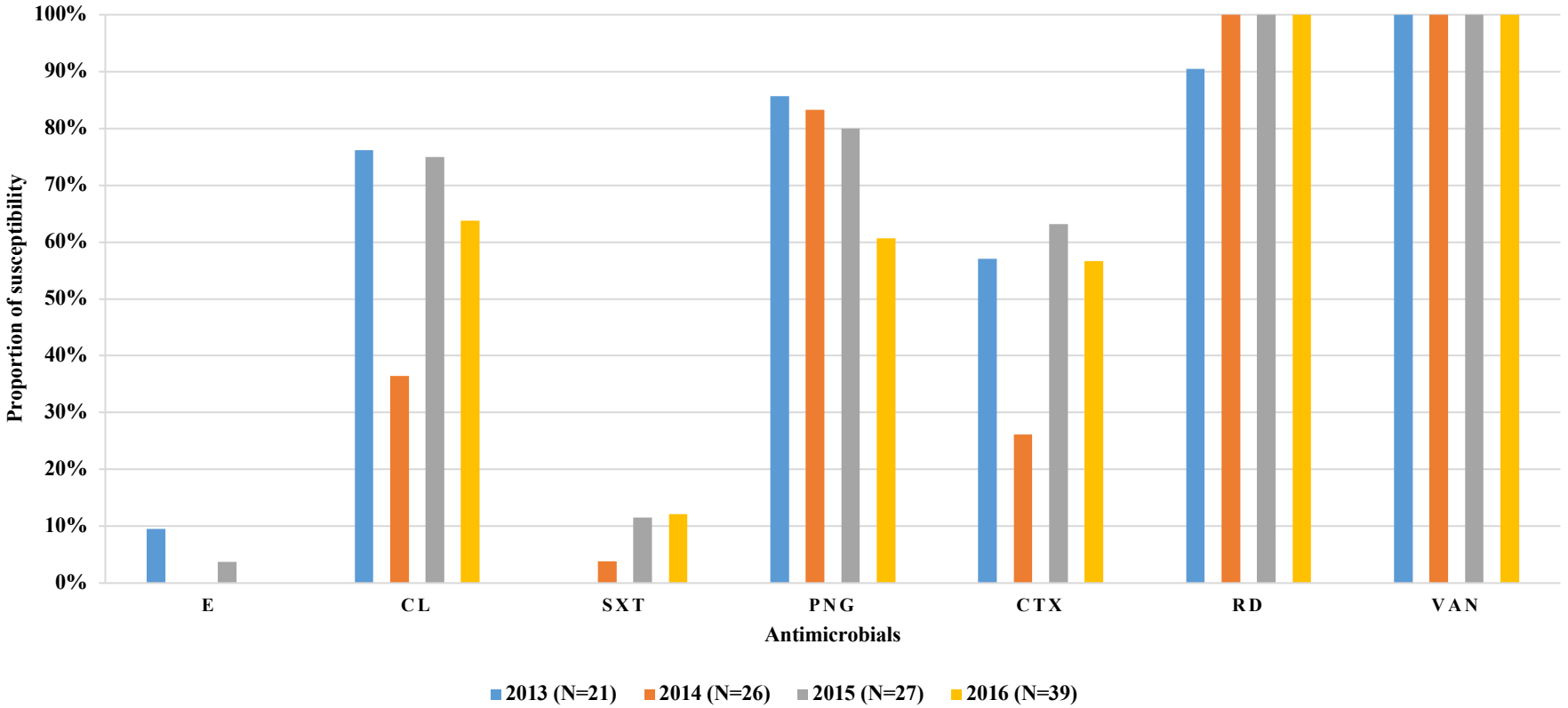

Figure 3.12 Antimicrobial susceptibility of *S. pneumoniae* isolated from blood culture of children at CH1 between 2013 and 2016
The antimicrobial susceptibility proportions of *S. pneumoniae* isolated from blood culture at CH1 in 2013 (blue), 2014 (orange), 2015 (grey), and 2016 (yellow). There was a significant decrease in the susceptibility of *S. pneumoniae* against penicillin G from 2013 to 2016. E: erythromycin, CL: chloramphenicol, SXT: trimethoprim-sulfamethoxazole, PNG: penicillin G, CTX: cefotaxime, RD: rifampicin, VAN: vancomycin.

3.2.13 Theoretical coverage of antimicrobials for BSIs between 2013 and 2016

There was considerable variability in the proportion of susceptible organisms isolated over the sampled years. I considered how antimicrobial therapy may change according to the proportion of specific antimicrobial susceptible organisms that may be isolated in a year. For example, in 2016, imipenem would theoretically have treated 61.1% of *Acinetobacter baumannii* infections, 88.0% of *Escherichia coli* infections, 57.7% of *Klebsiella* spp. infections, and 100% of *Pseudomonas* spp. infections. Using these data, it would suggest that imipenem would treat 57.7% of infections caused by these bacteria. Ultimately, the theoretical coverage of specific antimicrobials based on the proportions of susceptible isolated bacteria between 2013 and 2016 are shown in Table 3.2.

During the 4-year sampling period, the susceptibility of Gram-negative bacteria to imipenem ranged from 41.3% in 2015 to 57.7% in 2016. Vancomycin susceptibility was constantly highest at approximately 100% for Gram-positive bacteria over the entire period. However, the potential effectiveness of ampicillin, ceftazidime, cefepime, and oxacillin was <25%. This period also saw a significant reduction in the overall susceptibility to ciprofloxacin with 17.3% in 2016 (Figure 3.13).

Table 3.2 The theoretical coverage of antimicrobials for BSIs in children at CH1 between 2013 and 2016

Antimicrobials	2013 (%)	2014 (%)	2015 (%)	2016 (%)
Gentamicin	26.8	5.0	44.8	38.5
Ampicillin	25.0	12.3	8.8	6.0
Cefotaxime	17.4	9.5	31.4	48.3
Ceftazidime	22.2	22.9	19.0	7.7
Cefepime	24.4	20.0	20.7	23.5
Ciprofloxacin	26.1	23.8	45.2	17.3
Imipenem	56.5	72.1	41.3	57.7
Oxacillin	2.1	2.6	3.0	9.2
Vancomycin	100.0	100.0	99.5	99.8

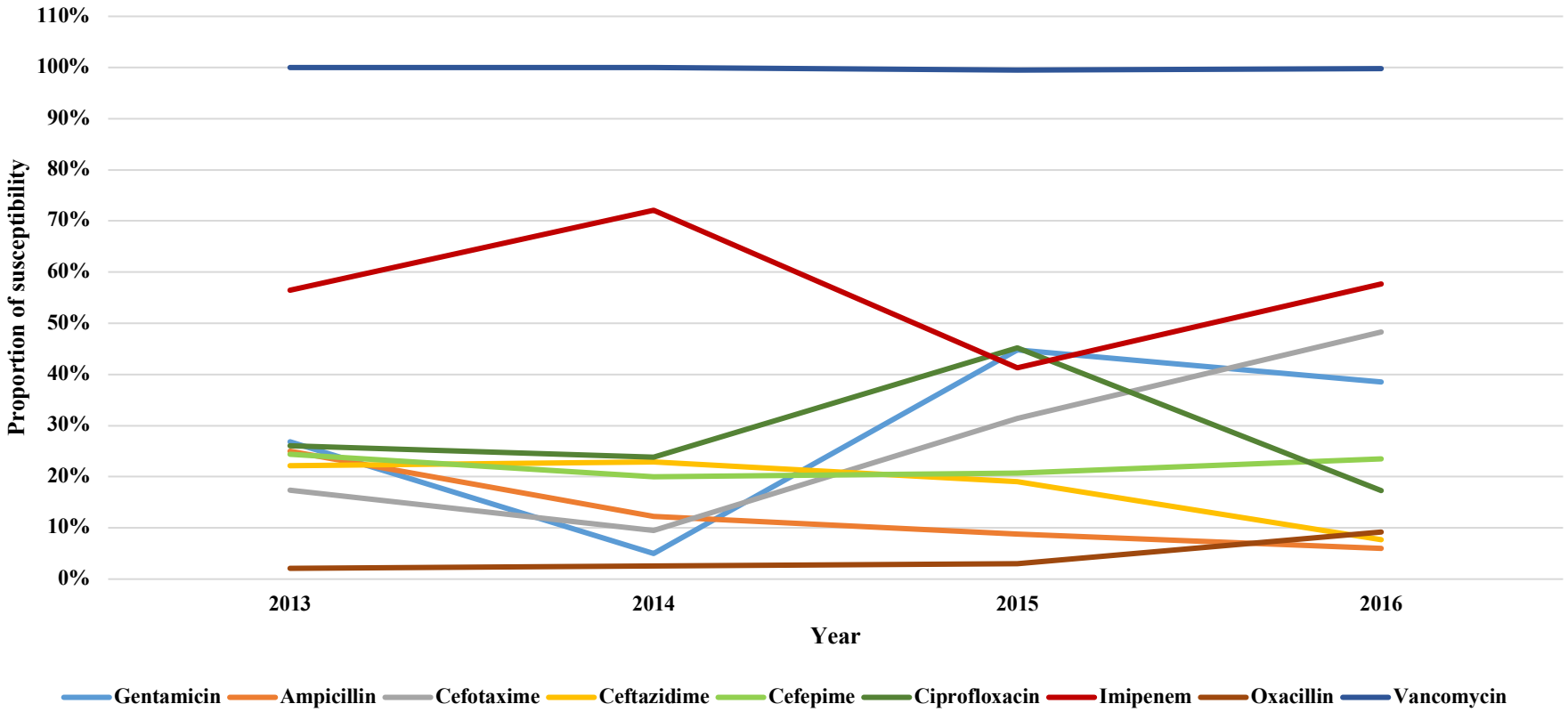

Figure 3.13 The theoretical coverage of empirical antimicrobials for BSIs in children at CH1 between 2013 and 2016

The theoretical coverage of specific antimicrobials based on the proportions of susceptible isolated bacteria between 2013 and 2016 are illustrated. Vancomycin (dark blue) and imipenem (red) theoretically showed the highest effectiveness in treating bacterial BSIs in children at CH1 during the 4-year period from 2013 to 2016. The potential coverage of ampicillin (orange), ceftazidime (yellow), cefepime (light green), and oxacillin (brown) for BSIs was <25%.

3.3 Discussion

This chapter provides some baseline data with respect to the bacterial aetiology of paediatric BSIs in our local setting over a 4-year period from 2013 to 2016. The positivity rate of blood cultures at CH1 ranged from 7.4% to 8.1% from 2013 to 2016. *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. were the most commonly isolated Gram-negative bacteria; while CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were the most commonly isolated Gram-positive organisms. The bacterial profile of BSIs in our study population were in accordance with those identified in other paediatric studies (169–175), especially investigations that focused on common pathogens including *Escherichia coli* (176,177), *Staphylococcus aureus* (178), and *Streptococcus pneumoniae* (179,180).

Our data were comparable with previous studies, in term of the major bacterial pathogens causing BSIs in children (29,181). A retrospective cohort study in the United States assessing demographic and microbiological data from hospitalised patients aged <19 years of age using the Premier Healthcare Database was conducted from 2009 to 2016 (181). From 5,340 patients with positive BSIs during the study period, 5,833 pathogens were isolated; the majority of pathogens (57%) were from non-neonates. The most common neonatal pathogens were *Escherichia coli* (22%), group B *Streptococcus* (21%), methicillin-sensitive *Staphylococcus aureus* (MSSA; 14%), *Enterococcus* spp. (10%), and *Klebsiella* spp. (7%). The most common non-neonatal pathogens were MSSA (16%), *Escherichia coli* (16%), *Streptococcus pneumoniae* (10%), MRSA (9%), *Enterococcus* spp. and *Klebsiella* spp. (6% each). From 2009 through 2016, statistically significant rises in the number of *E. coli* (0.88 to 1.26 per 1,000 admissions, p=0.0003), MSSA (0.83 to 1.12, p=0.02), and group A *Streptococcus* (0.16 to 0.37, p<0.001) isolated from non-

neonates, *S. pneumoniae* (1.07 to 0.26, $p<0.001$) and *Enterococcus* spp. (0.60 to 0.17, $p=0.01$) declined over the same period (181).

In SEA, data from a retrospective investigation of BSIs in children at a paediatric hospital and its satellite clinic in Siem Reap, Cambodia conducted from January 1, 2007, to July 31, 2011 were also consistent the data presented here (29). Among BSIs in children, *Staphylococcus aureus* (73/459 cases; 15.9%), *Streptococcus pneumoniae* (59/459; 12.9%), *Klebsiella pneumoniae* (37/459; 8.1%), *Escherichia coli* (35/459; 7.6%) and *Haemophilus influenzae* (28/459; 6.1%) were the most commonly isolated pathogen (29). However, the majority (81% of those identified to a genus level) were environmental glucose nonfermenting Gram-negative bacilli. *Acinetobacter baumannii–calcoaceticus* was isolated from 21 of 123 (17.1%) of these cases (29). Of first episodes of monomicrobial hospital-acquired BSIs, 37/53 (69.8%) were in children <1 year of age ($p<0.001$), with *Klebsiella pneumoniae* (41.5%), *Staphylococcus aureus* (13.5%) and *Acinetobacter baumannii–calcoaceticus* (13.5%) the most frequently cultured (29).

I observed a limited number of *Streptococcus pneumoniae*. *Streptococcus pneumoniae* remains the leading cause of infections in childhood globally, however, conjugate 7-valent and 13-valent vaccine has reduced the incidence of invasive *Streptococcus pneumoniae* infection by 76% (182,183). Additionally, *Staphylococcus aureus* was also uncommon. *Staphylococcus aureus* has become an increasingly common cause of infections and paediatric hospitalization (184). In intensive care units, with long lengths-of-stay, Gram-positive hospital-acquired pathogens, including CoNS and *Staphylococcus aureus* should be considered firstly during BSIs management (185,186).

Acinetobacter spp. showed resistance to many commonly used antimicrobials. In clinical practice, the susceptibility of *Acinetobacter* spp. to imipenem and meropenem ("last-resorts" drugs for the treatment of Gram-negative bacteraemia) decreased to only 61.1% and 51.6% in 2016, respectively. This phenomenon may threaten the current treatment of *Acinetobacter* spp. as our the commonly used class antimicrobials may not remain effective. Conversely, *Acinetobacter* spp. showed susceptibility to trimethoprim/sulfamethoxazole (56.1% in 2016), which is a "classical" antimicrobial that is no longer widely used. This observation suggests that we could add trimethoprim/sulfamethoxazole to our antimicrobial regime to treat sepsis caused by *Acinetobacter* spp.

During the 4-year period from 2013 to 2016, the overall susceptibility to ampicillin, ceftazidime, cefepime, and oxacillin decreased to <25% of all isolated bacteria. This is alarming, as ineffective beta-lactam antimicrobials may pose a further challenge to clinical practice. These results also indicated that ampicillin, ceftazidime, cefepime, and oxacillin would probably no longer be effective in treatment of BSIs at CH1. Ciprofloxacin was found to cover only 17.3% of isolated bacteria in 2016. However, our data suggested that vancomycin could be used treat all infections caused by Gram-positive bacteria. Additionally, imipenem would theoretically have treated almost half of all pathogens isolated from 2013 to 2016.

A change in the distribution of bacteria causing BSIs in children may be triggered by many factors. Advances in technology and laboratory diagnostic capacities have greatly improved the process of pathogen isolation and identification techniques. The introduction of vaccination, in which new vaccines, such as the pneumococcal and HIB

conjugate vaccines, have changed the epidemiology of numerous pathogens (179,180). In addition, local changes in environment and socio-economic status, both community- and hospital-level factors may lead to differences in pathogens causing BSIs (187). Strategies of infection prevention and control that successfully prevent one pathogen may lead to increases in other opportunistic pathogens (188). Finally, the use of medical devices outside the hospital and the pre-hospital administration of antimicrobials in children is increasing, which may increase the community-level exposure to pathogens and subsequent hospitalisations (185).

This study was limited by its observational design, retrospective bias, and non-systematic sampling of blood cultures that may underestimate the true burden of BSIs (189). Evaluating pre-hospital treatment is difficult, nevertheless, it is possible that many patients may have taken antimicrobials or been given intravenous fluids at home or in public/private clinics. The community use of antimicrobials may mislead blood culture results. Retrospective documentation may have limited the ability to identify the history of the diseases and the clinical and laboratory manifestation of patients. In addition, our study population included only paediatric patients admitted to one hospital, this may limit the generalisability of our findings to other settings. I could not obtain information on the precise method or volume of blood collected for culture, which may affect the sensitivity of blood culture.

3.4 Conclusion

Here, I described the bacterial profile of BSIs and highlighted the historic trends in AMR in CH1 from isolated bacteria from 2013 to 2016. Notably, BSIs at CH1 were commonly caused by major bacterial pathogens including *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae*, indicating that future research in CH1 should focus on these pathogens. Ampicillin, ceftazidime, cefepime, and oxacillin were not as effective in treating BSIs at CH1, as these antimicrobials were associated with low susceptibility rates. Imipenem and vancomycin should have remained active for treating infections caused by major bacterial pathogens at CH1.

4 The clinical features and factors associated with mortality of neonatal sepsis at Children's Hospital 1 in Ho Chi Minh City in Vietnam

4.1 Introduction

Infection is a leading cause of mortality and morbidity in neonates (1–3) and one of the common clinical presentations of infection during neonatal period is sepsis (17,94,119,190). Neonatal sepsis is a global health issue, with a global incidence estimated to be 2,202 (95% CI 1,099–4,360) for every 100,000 livebirths between 1979 and 2016 (6). These figures equate to ~3,000,000 cases of neonatal sepsis globally per year (6), with mortality ranging from 11% to 19% (6). Despite its severity, there is a lack of a consensus definition for neonatal sepsis (9,10). Although neonatal sepsis is broadly defined as a clinical syndrome with the presence of systemic inflammatory responses to a suspected or proven infection (classically based on the isolation of bacteria from a blood culture) occurring in new-borns, the variety of clinical and laboratory diagnostic criteria make the study of sepsis in neonates very challenging (79–81).

In LMICs, sepsis is one of the major causes of hospitalisation and death in neonates (144,191,192). Children born in LMICs settings are at a 3–20 times higher risk of severe neonatal infections than in higher-income countries (33). In previous studies from LMICs in SEA, neonatal sepsis was the most prevalent diagnosis at admission during a 3-year observation in East Timor, accounting for 38% of all admissions (25). Another study in Cambodia from 2007 to 2011 reported an overall mortality prevalence of 36.9% in neonatal BSIs (29). In Vietnam, neonatal sepsis was similarly observed as a common condition with a high associated mortality rate (26–28). A prospective study during a 12-

month period in the south of Vietnam reported a mortality of 16.1% (62/385) in neonates with BSIs (26). Furthermore, the overall case fatality rate for microbiologically confirmed sepsis among 616 neonatal patients was an alarming 46% from data of a prospective cohort study in the largest neonatal unit in central Vietnam (27,28).

The majority of guidelines for the management of neonatal sepsis have been developed in high-income countries (91–93), however, these guidelines are difficult to transpose to complex clinical settings and less well-resourced healthcare facilities in LMICs (94–96). Moreover, insufficient clinical assessments and a delay in the identification of sepsis cases impact on current management strategies. More specifically, we have inadequate clinical predictors of poor outcome due to limited or non-existent local surveillance systems in LMICs (95,96). Data that are available found that mortality was higher in Filipino neonates <1 week of age in those with dense or diffuse infiltrates in chest X-ray (53), and a retrospective study in Cambodia suggested that meningitis or meningoencephalitis was independently associated with mortality (29).

There are limited current data about the clinical features of bacterial neonatal sepsis and the factors associated with mortality in Vietnam. Understanding the clinical features of sepsis in neonatal population is essential if we are to identify the sepsis patients early, in order to plan strategies to improve outcomes. In addition, early recognition of warning signs would allow more timely evaluation, recognition of at-risk patients, the development of a risk-based management approach, and more effective treatment. Here, I aimed to investigate the features and outcome of neonatal sepsis; and to identify the clinical and laboratory observations associated with mortality of neonates with sepsis at CH1 in HCMC in Vietnam.

4.2 Results

4.2.1 Baseline characteristics of neonates with sepsis at CH1

Over the 18-month study period (January 2017 to June 2018), there were 8,497 neonates admitted to CH1. There were 5,472 blood cultures conducted in 8,497 neonatal admissions. Among 8,497 neonatal admissions, 524 patients were enrolled into this neonatal sepsis study. The proportion of sepsis amongst the total number of admissions was 6.2% (524 neonatal sepsis cases/8,497 neonatal admissions). Among these 524 patients, 180 patients were diagnosed as probable sepsis (180/524, 34.4%) and 344 patients were diagnosed as culture-confirmed sepsis (34/524, 65.6%). There were 3,091 blood cultures conducted in these 524 neonatal sepsis patients. The positivity rate of blood cultures was 15.2% (469 bacterial isolates/3,091 blood cultures). Contaminants were identified in 64/3,091 blood cultures (2.1%). There were 405 bacterial pathogens isolated from 344 culture-confirmed sepsis patients (Figure 4.1). The overall incidence of neonatal sepsis was 61.7 per 1,000 neonatal admissions.

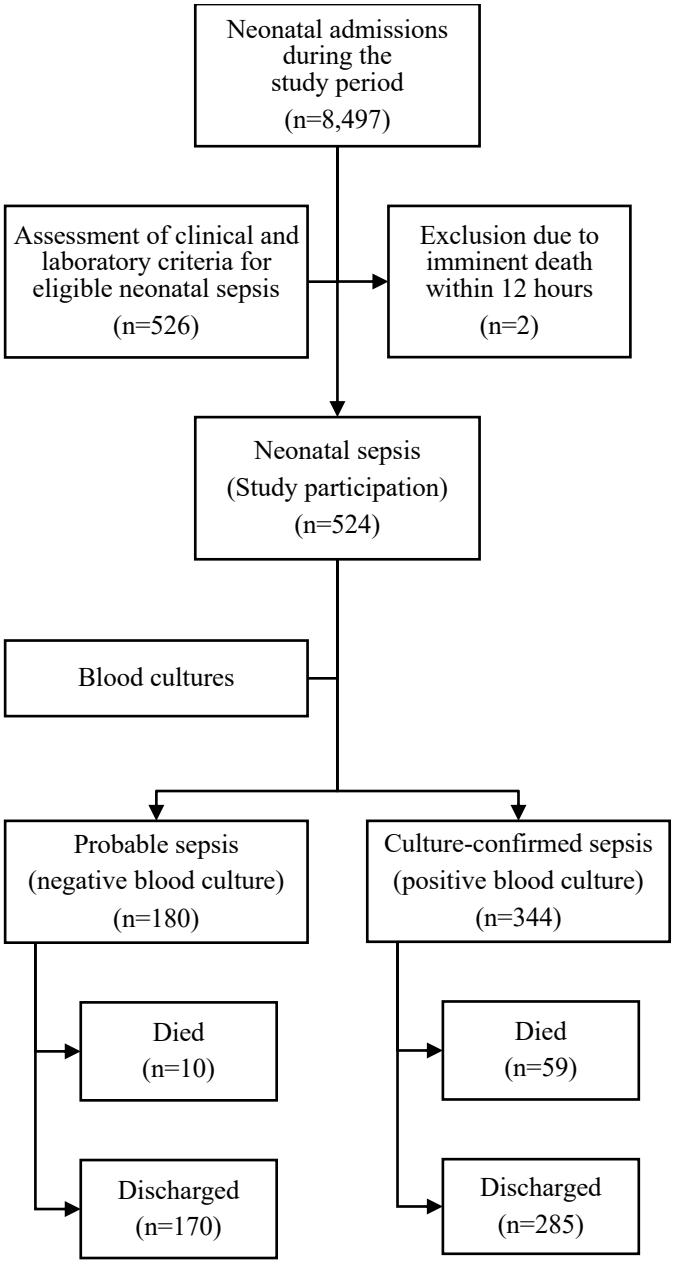

Figure 4.1 Flow chart for the clinical study of neonatal sepsis at CH1

A total of 8,497 neonates were admitted to CH1 during the study period. After assessment of clinical and laboratory diagnostic criteria for the diagnosis of sepsis, 526 patients were eligible for the study of which 2 patients were excluded due to imminent death within 2 hours. Totally 524 neonates were recruited into the study of neonatal sepsis. Culture-confirmed sepsis was diagnosed in 344/524 neonates. Death was found in 69/524 neonates with sepsis.

Between January 2017 and June 2018, I enrolled 524 neonates in this single healthcare facility with a diagnosis of probable sepsis that met the inclusion criteria. The baseline characteristics of recruited neonates diagnosed with probable sepsis are shown in Table 4.1.

The male to female patient ratio was 1.6:1 and the median age of gestation was 38 weeks (IQR; 33–40 weeks). A high proportion (38.5%) were born prematurely, this was reflected in a similar proportion (42.6%) having a low birth weight; median birth weight 2,700 grams (IQR; 1,800–3,200 grams). The prevalence of Caesarean section delivery in this group of patients was 36.3% (190/524), but other factors often considered to be associated with neonatal sepsis (perinatal asphyxia, intrapartum fever >38^{o}C, chorioamnionitis, and the rupture of membranes >18 hours) were relatively uncommon. Notably, 57/524 (10.9%) of mothers reported using a maternal hot bed (a local custom that involves providing additional heat under the child's bed during the early days of life). The majority of cases were determined to be LOS and HAS (91.4% and 73.3%, respectively; Table 4.1). The incidence of HAS was calculated as 42.2 per 1,000 neonatal admissions (4.2%).

Table 4.1 Baseline characteristics of 524 neonates with sepsis at CH1

	n (%) or median (IQR)
Demographic features	
Male	320 (61.1)
Female	204 (38.9)
Gestational age (weeks)	38 (33–40)
Prematurity	202 (38.5)
Birth weight (grams)	2,700 (1,800–3,200)
Low birth weight	223 (42.6)
Maternal features	
Vaginal delivery	334 (63.7)
C-section delivery	190 (36.3)
Perinatal asphyxia	14 (2.7)
Intrapartum fever >38^{o}C	4 (0.8)
Chorioamnionitis	13 (2.5)
Rupture of membranes >18 hours	8 (1.5)
Maternal hot bed	57 (10.9)
Diagnosis of sepsis	
Probable sepsis	180 (34.4)
Culture-confirmed sepsis	344 (65.6)
Onset of sepsis	
EOS	45 (8.6)
LOS	479 (91.4)
Acquisition of sepsis	
CAS	140 (26.7)
HAS	384 (73.3)

4.2.2 Clinical features of neonatal sepsis at CH1

The clinical features of sepsis this neonatal were highly variable and often nonspecific. However, some common factors in this patient cohort and the key observations of 524 patients recruited with neonatal sepsis at CH1 are highlighted in Table 4.2. Specifically, clinical features were focused on the gastrointestinal system (47.5%; 249/524 exhibited feeding intolerance), the respiratory system (36.5%; 191/524 cases required mechanical ventilation), fever (32.8%; 172/524), severe jaundice (26.0%; 136/524 of cases), the cardiovascular system (16.4%; 86/524 cases had impaired peripheral perfusion), and neurological manifestations (13.7%; 72/524 had lethargy) (Table 4.2).

Table 4.2 Key observations of 524 patients recruited with neonatal sepsis at CH1

	n (%) or median (IQR)
Clinical features	
Fever >38.5^{o}C	172 (32.8)
Requirement for mechanical ventilation	191 (36.5)
Impaired peripheral perfusion	86 (16.4)
Feeding intolerance	249 (47.5)
Lethargy	72 (13.7)
Severe jaundice	136 (26.0)
Laboratory results	
Leukopenia <4,000/mm^3	29 (5.5)
Leucocytosis >20,000/mm^3	206 (39.3)
Thrombocytopenia <100,000/mm^3	135 (25.8)
CRP (mg/L)	23.9 (3.5–58.3)
CRP >15 mg/L	319 (60.9)
Metabolic acidosis	182 (34.7)
Base excess (mEq/L)	–15 (–11.9 to –19.1)
Base excess < –20 mEq/L	37 (7.1)
Serum lactate (mmol/L)	4.9 (2.5–8.7)
Serum lactate >4 mmol/L	33 (6.3)
Treatment	
Inappropriate empirical antimicrobial use	261 (261/344, 75.9%)
Duration of mechanical ventilation (days)	5.0 (2.0–10.0)
Duration of total parenteral nutrition (days)	11.0 (6.0–21.0)
Duration of lipid infusion (days)	18.0 (11.0–27.0)
Duration of central lines (days)	21.0 (7.0–21.0)
Surgical/invasive intervention	171 (32.6)
Shock management	90 (17.2)
Blood products transfusion	227 (43.3)
Antacids	109 (20.8)
Corticosteroids	13 (2.5)
Severity	
NTISS	17 (11–27)
NTISS >10	399 (76.1)
Severe sepsis	121 (23.1)
Septic shock	73 (13.9)
Outcomes	
Duration of stay (days)	23 (13–41)
Mortality	69 (13.2)

The presentation of neonatal sepsis in this study ranged from mild or moderate manifestations, to severe sepsis (23.1%; 121/524), and septic shock (13.9%; 73/524). The median (IQR) severity score using NTISS was 17 (11–27), and 399 cases (76.1%) had a NTISS severity score above the low severity threshold of 10. The median (IQR) duration of hospital stay was 23 (13–41) days and mortality was recorded for 69/524 (13.2%) study participants (Table 4.2).

4.2.3 Laboratory features of neonatal sepsis at CH1

Blood samples from all patients were subjected to a complete blood count, C-reactive protein (CRP), and blood culture (Table 4.2). I found that 29/524 (5.5%) cases had leukopenia <4,000/mm^3 and 206/524 (39.3%) cases had leukocytosis >20,000/mm^3. The median (IQR) CRP concentration was 23.9 (3.5–58.3) mg/L and 319 (60.9%) patients had a CRP >15 mg/L. In addition, 182/524 (34.7%) patients had evidence of metabolic acidosis, which was often severe with a median (IQR) base excess of –15 (–11.9 to –19.1) mEq/L. High lactate level was also observed with 33/524 (6.3%) patients had a value of serum lactate >4 mmol/L, and the median (IQR) serum lactate value being 4.9 (2.5–8.7) mmol/L (Table 4.2). Ultimately, two thirds (65.6%; 344/524) of cases had a positive blood culture and were categorised as culture-confirmed sepsis (Table 4.1).

4.2.4 CRP cut-off points for the prediction of a positive blood culture

Five different cut-off points of CRP (>10 mg/L, >15 mg/L, >20 mg/L, >25 mg/L, and >30 mg/L) were analysed for the prediction of a positive blood culture. The receiver operating characteristic (ROC) areas of CRP cut-off points >10 mg/L, >15 mg/L, >20 mg/L, >25 mg/L, and >30 mg/L were 0.4183, 0.4142, 0.4322, 0.4669, and 0.4754; respectively (Figure 4.2), showing that CRP is poor predictor of a positive blood culture.

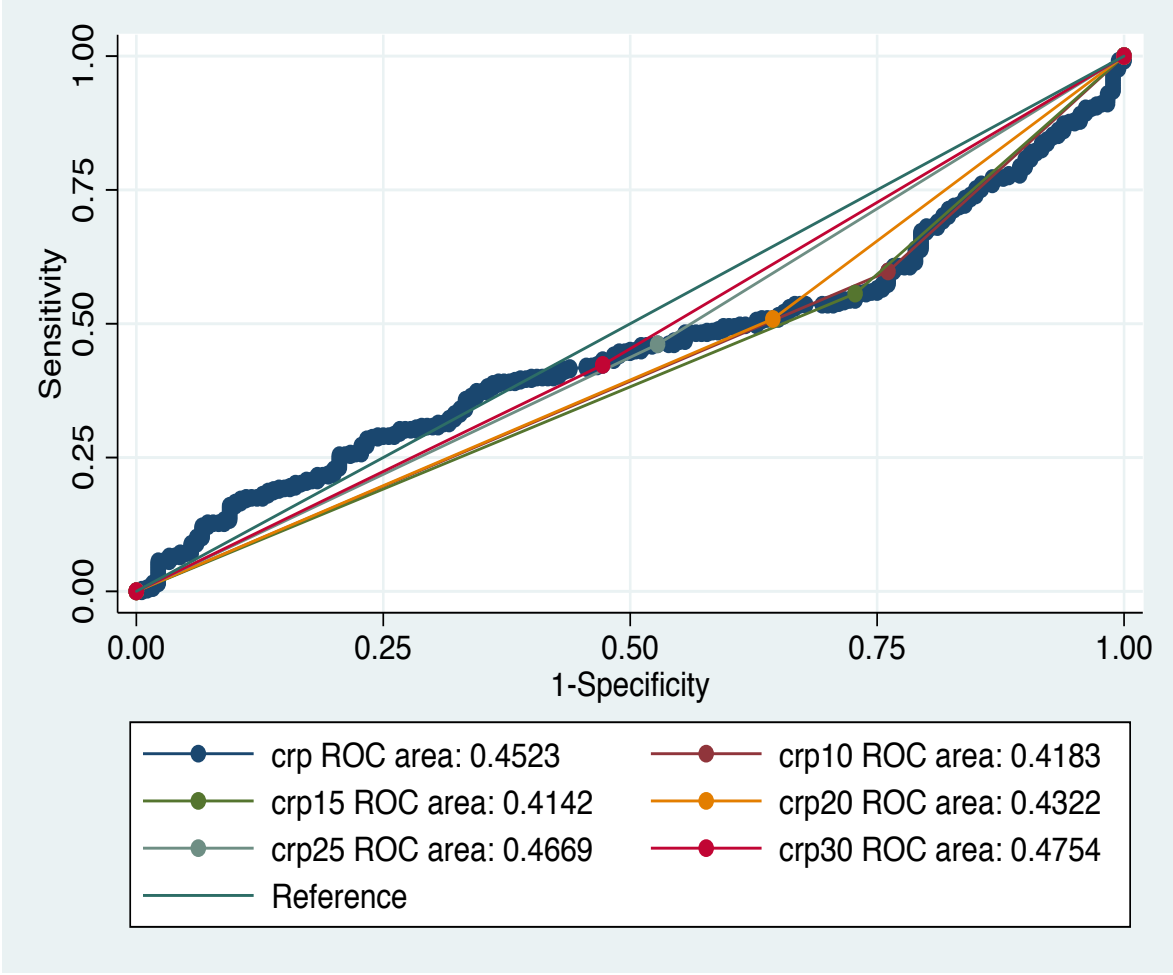

Figure 4.2 ROC curves of different CRP cut-off points for the prediction of a positive blood culture

The cut-off points of CRP including >10 mg/L, >15 mg/L, >20 mg/L, >25 mg/L, and >30 mg/L were chosen to optimise the sensitivity and the specificity of CRP in predicting a positive blood culture. The area under the curve was calculated for each ROC curve. A ROC area value of 0.5 represents a random prediction, and thus all ROC areas of CRP cut-off points <0.5 were considered low and represent poor prediction of a positive blood culture.

4.2.5 Treatment of neonatal sepsis at CH1

Requirement for mechanical ventilation was recorded in 191/524 cases (36.5%). The median (IQR) duration of mechanical ventilation and total parenteral nutrition were 5.0 (2.0–10.0) and 11.0 (6.0–21.0) days, respectively. The median (IQR) durations of lipid infusion and central lines were 18.0 (11.0–27.0) and 21.0 (7.0–21.0) days, respectively. A surgical intervention or invasive procedure was performed in 171/524 (32.6%) cases, shock management was implemented in 90/524 (17.2%) cases, and blood products transfusion was given in 227/524 (43.3%) cases (Table 4.2).

4.2.6 Factors associated with mortality of neonates with sepsis at CH1

A comparison of demographic features, maternal factors, clinical characteristics, laboratory results, diagnoses, treatments, and outcomes between the neonatal sepsis patients who died and survived are shown in Table 4.3. A univariable analysis revealed a number of key factors including extreme prematurity, extremely low birth weight, culture-confirmed sepsis, HAS, hypothermia <36^{o}C, mottled skin, mechanical ventilation, feeding intolerance, sclerema, lethargy, leukopenia <4,000/mm^{3}, thrombocytopenia <100,000/mm^{3}, hyperglycaemia >180 mg/dL, base excess < –20 mEq/L, serum lactate >4 mmol/L, score of severity (NTISS), severe sepsis, and septic shock were significantly different between the deceased group and the survival group (Table 4.3).

Table 4.3 Univariable analysis for factors associated with mortality of neonates with sepsis at CH1

	Died (n=69)	Survived (n=455)	*P*-values*
Demographic features			
Extreme prematurity	12 (17.4)	17 (3.7)	**<0.001**
Extremely low birth weight	10 (14.5)	17 (3.7)	**<0.001**
Maternal features			
C-section delivery	23 (33.3)	167 (36.7)	0.890
Perinatal asphyxia	2 (2.9)	12 (2.6)	0.900
Intrapartum fever >38^{o}C	1 (1.4)	3 (0.7)	0.482
Chorioamnionitis	1 (1.4)	12 (2.6)	0.554
Rupture of membranes >18 hours	3 (4.3)	5 (1.1)	**0.040**
Neonatal sepsis classification			
Culture-confirmed sepsis	59 (85.5)	285 (62.6)	**<0.001**
LOS	61 (88.4)	418 (91.9)	0.339
HAS	67 (97.1)	317 (69.7)	**<0.001**
Clinical manifestations			
Fever >38.5^{o}C	12 (17.4)	160 (35.2)	**0.003**
Hypothermia <36^{o}C	30 (43.5)	65 (14.3)	**<0.001**
Temperature instability	26 (37.7)	36 (7.9)	**<0.001**
Bradycardia	8 (11.6)	17 (3.7)	**0.004**
Tachycardia	5 (7.2)	7 (1.5)	**0.003**
Rhythm instability	3 (4.3)	5 (1.1)	**0.040**
Reduced urinary output	10 (14.5)	1 (0.2)	**<0.001**
Hypotension	46 (66.7)	27 (5.9)	**<0.001**
Mottled skin	44 (63.8)	40 (8.8)	**<0.001**
Impaired peripheral perfusion	45 (65.2)	41 (9.0)	**<0.001**
Apnoea episodes	33 (47.8)	54 (11.9)	**<0.001**
Bradypnoea	2 (2.9)	3 (0.7)	0.075
Tachypnoea	1 (1.4)	8 (1.8)	0.854
Increased oxygen requirements	67 (97.1)	280 (61.5)	**<0.001**
Mechanical ventilation	62 (89.9)	129 (28.4)	**<0.001**
Feeding intolerance	60 (87.0)	189 (41.5)	**<0.001**
Poor sucking	58 (84.1)	234 (51.4)	**<0.001**
Abdominal distension	42 (60.9)	138 (30.3)	**<0.001**
Petechial rash	13 (18.8)	5 (1.1)	**<0.001**
Sclerema	16 (23.2)	2 (0.4)	**<0.001**
Irritability	0 (0.0)	4 (0.9)	1.000
Lethargy	24 (34.8)	48 (10.5)	**<0.001**
Hypotonia	20 (29.0)	21 (4.6)	**<0.001**
Seizure	3 (4.3)	13 (2.9)	0.502
Severe jaundice	24 (34.8)	112 (24.6)	0.073
Laboratory findings			
Leukopenia <4,000/mm^3	16 (23.2)	13 (2.9)	**<0.001**

Values are n (%) or median (IQR), *Wilcoxon rank-sum test, Chi-squared test or Fisher's exact test.

Table 4.3 Univariable analysis for factors associated with mortality of neonates with sepsis at CH1 (continued)

	Died (n=69)	Survived (n=455)	*P*-values*
Leucocytosis >20,000/mm^3	33 (47.8)	173 (38.0)	0.120
Thrombocytopenia <100,000/mm^3	43 (62.3)	92 (20.2)	**<0.001**
CRP >15 mg/L	51 (73.9)	268 (58.9)	**0.017**
Hypoglycaemia <45 mg/dL	15 (21.7)	40 (8.8)	**0.001**
Hyperglycaemia >180 mg/dL	21 (30.4)	34 (7.5)	**<0.001**
Base excess < –20 mEq/L	21 (30.4)	16 (3.5)	**<0.001**
Serum lactate >4 mmol/L	14 (20.3)	19 (4.2)	**<0.001**
Total bilirubin (µmol/L)	166.8 (103.8–225.2)	173.8 (127.0–224.7)	0.416
Creatinine (µmol/L)	63.8 (42.2–125.6)	53.1 (40.6–72.9)	**0.002**
Alanine aminotransferase (IU/L)	13 (8–35)	14 (9–23)	0.744
Electrolyte disturbance	51 (73.9)	113 (24.8)	**<0.001**
Abnormal coagulation	23 (33.3)	20 (4.4)	**<0.001**
Infection conditions/syndromes			
Pneumonia	51 (73.9)	259 (56.9)	**0.007**
Catheter-related bloodstream infection	29 (42.0)	80 (17.6)	**<0.001**
Necrotizing enterocolitis	14 (20.3)	88 (19.3)	0.853
Meningitis	14 (20.3)	84 (18.5)	0.717
Peritonitis	10 (14.5)	19 (4.2)	**<0.001**
Urinary infection	2 (2.9)	4 (0.9)	0.142
Comorbidities			
Congenital heart diseases	34 (49.3)	97 (21.3)	**<0.001**
Congenital gastrointestinal anomalies	18 (26.1)	80 (17.6)	0.091
Respiratory distress syndrome	13 (18.8)	45 (9.9)	**0.027**
Bronchopulmonary dysplasia	10 (14.5)	15 (3.3)	**<0.001**
Pulmonary hypertension	9 (13.0)	14 (3.1)	**<0.001**
Congenital renal anomalies	3 (4.3)	4 (0.9)	**0.019**
Treatment			
Inappropriate empirical antimicrobials	39/59 (66.1%)	222/285 (77.9%)	0.054
Duration of mechanical ventilation (days)	8 (3–21)	4 (2–7)	**<0.001**
Duration of parenteral nutrition (days)	14 (7–40)	11 (6–19)	**0.013**
Duration of lipid infusion (days)	14 (5–38)	19 (12–23)	0.830
Duration of central catheters (days)	21 (11–21)	21 (7–21)	0.255
Surgical/invasive intervention	37 (53.6)	134 (29.5)	**<0.001**
Shock management	55 (79.7)	35 (7.7)	**<0.001**
Blood products transfusion	57 (82.6)	170 (37.4)	**<0.001**
Antacids	9 (13.0)	100 (22.0)	0.088
Corticosteroids	4 (5.8)	9 (2.0)	0.057
Severity and duration of stay			
Score of severity (NTISS)	34 (25–40)	15 (10–24)	**<0.001**
Severe sepsis	56 (81.2)	65 (14.3)	**<0.001**
Septic shock	46 (66.7)	27 (5.9)	**<0.001**
Duration of stay (days)	29 (8–64)	23 (13–38)	0.365

Values are n (%) or median (IQR), *Wilcoxon rank-sum test, Chi-squared test or Fisher's exact test.

To identity factors associated with mortality I performed a multivariable logistic regression analysis with seven factors: extremely low birth weight; sclerema; leukopenia <4,000/mm^3; thrombocytopenia <100,000/mm^3; hyperglycaemia >180 mg/dL; base excess <–20 mEq/L; and serum lactate >4 mmol/L. The selection of these seven variables was based on clinical judgement and the results of previous studies (193–202). I found sequentially that sclerema (OR=11.4; 95% CI, 2.0–63.1; p=0.005), leukopenia <4,000/mm^3 (OR=7.8; 95% CI, 2.9–20.8; p<0.001), thrombocytopenia <100,000/mm^3 (OR=3.7; 95% CI, 1.9–7.0; p<0.001), base excess < –20 mEq/L (OR=3.6; 95% CI, 1.3–9.8; p=0.012), serum lactate >4 mmol/L (OR=3.4; 95% CI, 1.2–9.1; p=0.015), extremely low birth weight (OR=3.2; 95% CI, 1.1–9.0; p=0.022), and hyperglycaemia >180 mg/dL (OR=2.6; 95% CI, 1.1–6.1; p=0.021) were all significantly associated with mortality (Table 4.4).

Table 4.4 Multivariable analysis for factors associated with mortality of neonates with sepsis at CH1

Mortality-associated factors	OR*	95% CI*	*P*-values*
Sclerema	11.4	2.0–63.1	**0.005**
Leukopenia <4,000/mm^3	7.8	2.9–20.8	**<0.001**
Thrombocytopenia <100,000/mm^3	3.7	1.9–7.0	**<0.001**
Base excess < –20 mEq/L	3.6	1.3–9.8	**0.012**
Serum lactate >4 mmol/L	3.4	1.2–9.1	**0.015**
Extremely low birth weight	3.2	1.1–9.0	**0.022**
Hyperglycaemia >180 mg/dL	2.6	1.1–6.1	**0.021**

*****Logistic regression analysis. OR: odds ratio, CI: confidence interval.

I additionally performed a lasso regression analysis to identify further variables associated with mortality. Extremely low birth weight was rejected in this model, but the other six factors remained significantly associated with mortality, with sclerema exhibiting the most significant association (Table 4.5).

Table 4.5 Estimation of factors associated with mortality of neonatal sepsis

Mortality-associated factors	Lasso*	Post-est OLS*
Sclerema	0.365	0.440
Leukopenia <4,000/mm^3	0.132	0.292
Base excess < –20 mEq/L	0.092	0.169
Thrombocytopenia <100,000/mm^3	0.076	0.148
Hyperglycaemia >180 mg/dL	0.021	0.128
Serum lactate >4 mmol/L	0.001	0.138

*lasso regression analysis, using λ-lse = 53.789, the largest lambda (λ) value for which the mean-squared prediction error (MSPE) is within one standard error of the minimal MSPE. OLS: ordinary least squares.

4.2.7 Comparisons of CAS and HAS in neonates at CH1

A univariable analysis revealed a number of factors that were significantly different between the CAS group and the HAS group (Table 4.6). Factors including culture-confirmed sepsis, fever >38.5ºC, hypothermia <36ºC, temperature instability, hypotension, impaired peripheral perfusion, apnoea episodes, increased oxygen requirements, mechanical ventilation, feeding intolerance, poor sucking, abdominal distension, hypotonia, thrombocytopenia <100,000/mm^3, hypoglycaemia <45 mg/dL, electrolyte disturbance, pneumonia, catheter-related bloodstream infection, congenital heart diseases, congenital gastrointestinal anomalies, respiratory distress syndrome, duration of parenteral nutrition, surgical/invasive intervention, shock management, blood products transfusion, score of severity (NTISS), severe sepsis, septic shock, and duration of stay were found to be significantly different between CAS and HAS using univariable analysis (p<0.001).

Table 4.6 Comparisons of CAS and HAS in neonates at CH1

	CAS (n=140)	HAS (n=384)	*P*-values*
Demographic features			
Extreme prematurity	1 (0.7)	28 (7.3)	**0.004**
Extremely low birth weight	3 (2.1)	24 (6.3)	0.060
Maternal features			
C-section delivery	50 (35.7)	140 (36.5)	0.856
Perinatal asphyxia	0 (0.0)	14 (3.6)	**0.026**
Intrapartum fever >38ºC	0 (0.0)	4 (1.0)	0.578
Chorioamnionitis	0 (0.0)	13 (3.4)	**0.025**
Rupture of membranes >18 hours	1 (0.7)	7 (1.8)	0.360
Neonatal sepsis classification			
Culture-confirmed sepsis	72 (51.4)	272 (70.8)	**<0.001**
LOS	137 (97.9)	342 (89.1)	**0.001**
Clinical manifestations			
Fever >38.5ºC	86 (61.4)	86 (22.4)	**<0.001**
Hypothermia <36ºC	8 (5.7)	87 (22.7)	**<0.001**
Temperature instability	1 (0.7)	61 (15.9)	**<0.001**
Bradycardia	2 (1.4)	23 (6.0)	**0.030**
Tachycardia	5 (3.6)	7 (1.8)	0.236
Rhythm instability	1 (0.7)	7 (1.8)	0.360
Reduced urinary output	1 (0.7)	10 (2.6)	0.182
Hypotension	5 (3.6)	68 (17.7)	**<0.001**
Mottled skin	10 (7.1)	74 (19.3)	**0.001**
Impaired peripheral perfusion	8 (5.7)	78 (20.3)	**<0.001**
Apnoea episodes	9 (6.4)	78 (20.3)	**<0.001**
Bradypnoea	0 (0.0)	5 (1.3)	0.331
Tachypnoea	2 (1.4)	7 (1.8)	0.759
Increased oxygen requirements	43 (30.7)	304 (79.2)	**<0.001**
Mechanical ventilation	11 (7.9)	180 (46.9)	**<0.001**
Feeding intolerance	21 (15.0)	228 (59.4)	**<0.001**
Poor sucking	44 (31.4)	248 (64.6)	**<0.001**
Abdominal distension	24 (17.1)	156 (40.6)	**<0.001**
Petechial rash	3 (2.1)	15 (3.9)	0.327
Sclerema	0 (0.0)	18 (4.7)	**0.005**
Irritability	2 (1.4)	2 (0.5)	0.291
Lethargy	19 (13.6)	53 (13.8)	0.946
Hypotonia	1 (0.7)	40 (10.4)	**<0.001**
Seizure	4 (2.9)	12 (3.1)	0.875
Severe jaundice	28 (20.0)	108 (28.1)	0.060
Laboratory findings			
Leukopenia <4,000/mm^3	4 (2.9)	25 (6.5)	0.106

Values are n (%) or median (IQR), *Wilcoxon rank-sum test, Chi-squared test or Fisher's exact test.

Table 4.6 Comparisons of CAS and HAS in neonates at CH1 (continued)

	CAS (n=140)	HAS (n=384)	*P*-values*
Leucocytosis >20,000/mm^3	54 (38.6)	152 (39.6)	0.834
Thrombocytopenia <100,000/mm^3	14 (10.0)	121 (31.5)	**<0.001**
CRP >15 mg/L	88 (62.9)	231 (60.2)	0.575
Hypoglycaemia <45 mg/dL	3 (2.1)	52 (13.5)	**<0.001**
Hyperglycaemia >180 mg/dL	4 (2.9)	51 (13.3)	**0.001**
Base excess < –20 mEq/L	6 (4.3)	31 (8.1)	0.134
Serum lactate >4 mmol/L	2 (1.4)	31 (8.1)	**0.006**
Total bilirubin (μmol/L)	202.5 (158.0–243.2)	168.2 (112.3–219.3)	**0.016**
Creatinine (μmol/L)	44.8 (38.2–58.3)	58.1 (42.3–82.2)	**<0.001**
Alanine aminotransferase (IU/L)	17 (11–23)	14 (9–24)	0.325
Electrolyte disturbance	22 (15.7)	142 (37.0)	**<0.001**
Abnormal coagulation	3 (2.1)	40 (10.4)	**0.002**
Infection conditions/syndromes			
Pneumonia	56 (40.0)	254 (66.1)	**<0.001**
Catheter-related bloodstream infection	6 (4.3)	103 (26.8)	**<0.001**
Necrotizing enterocolitis	31 (22.1)	71 (18.5)	0.350
Meningitis	28 (20.0)	70 (18.2)	0.646
Peritonitis	2 (1.4)	27 (7.0)	**0.013**
Urinary infection	0 (0.0)	6 (1.6)	0.350
Comorbidities			
Congenital heart diseases	9 (6.4)	122 (31.8)	**<0.001**
Congenital gastrointestinal anomalies	5 (3.6)	93 (24.2)	**<0.001**
Respiratory distress syndrome	3 (2.1)	55 (14.3)	**<0.001**
Bronchopulmonary dysplasia	4 (2.9)	21 (5.5)	0.215
Pulmonary hypertension	1 (0.7)	22 (5.7)	**0.013**
Congenital renal anomalies	0 (0.0)	7 (1.8)	0.198
Treatment			
Duration of mechanical ventilation (days)	4 (2–6)	5 (2–11)	0.252
Duration of parenteral nutrition (days)	6.0 (2.5–9.0)	13.0 (7.0–24.0)	**<0.001**
Duration of lipid infusion (days)	3.5 (3.0–4.0)	19.0 (12.0–28.0)	**0.033**
Duration of central catheters (days)	13.0 (4.5–17.5)	21.0 (7.0–21.0)	0.099
Surgical/invasive intervention	17 (12.1)	154 (40.1)	**<0.001**
Shock management	5 (3.6)	85 (22.1)	**<0.001**
Blood products transfusion	27 (19.3)	200 (52.1)	**<0.001**
Antacids	23 (16.4)	86 (22.4)	0.136
Corticosteroids	1 (0.7)	12 (3.1)	0.116
Severity and duration of stay			
Score of severity (NTISS)	10 (8–13)	22 (14–30)	**<0.001**
Severe sepsis	9 (6.4)	112 (29.2)	**<0.001**
Septic shock	5 (3.6)	68 (17.7)	**<0.001**
Duration of stay (days)	16 (9–23)	28 (16–49)	**<0.001**

Values are n (%) or median (IQR), *Wilcoxon rank-sum test, Chi-squared test or Fisher's exact test.

4.3 Discussion

Here, I presented data from an observational study of 524 neonates with sepsis recruited at a single centre in southern Vietnam. The male to female ratio was ~1.6:1. This ratio was similar to those observed in other studies but the reason for the higher proportion of sepsis among male populations has not been determined (203). Studies have suggested that female patients had a greater degree of protection due to hormonal and immunological interactions during the course of sepsis, whereas male gender may be more susceptible (203). In this study, the median of gestational age was 38 weeks, with an IQR from 33 weeks to 40 weeks. The incidence of neonatal sepsis has been found to inversely related to gestational age (23). Premature neonates are at higher risk for sepsis than full-term patients due to low levels of circulating maternal immunoglobulin G (IgG), and the reduced function of neonatal skin and mucous barriers (204). The median of birth weight was 2,700 grams with the value at 25^{th} percentile was 1,800 grams and the value at 75^{th} percentile was 3,200 grams. Neonatal sepsis contributes greatly to the morbidity and mortality of low birthweight new-born infants, this observation was illustrated in a study of the previously mentioned National Institute of Child Health and Human Development (NICHD) registry (17). These data should prompt clinicians that sepsis should be cautiously investigated in the preterm and low birthweight neonates as this population are greatly impacted by sepsis.

Among 524 recruited neonates, I found various maternal/obstetrical factors that were associated with neonatal sepsis. All identified factors are known as risk factors for EOS in new-born infants (205). In term of perinatal asphyxia, resuscitation in the delivery room may increases risks of bacterial sepsis (206). Chorioamnionitis has been shown to be an early sign of intrauterine infection predisposing sepsis in the new-born (206). The risk of

confirmed sepsis may increase 10-fold to 1% in cases having prolonged rupture of membrane >18 hours (206). Maternal and obstetrical features should be considered when clinically assessing risk factors of sepsis in any neonates. These factors increase the risk of sepsis, as these interventions are likely to increase exposure to pathogens during pregnancy, delivery, or resuscitation at birth (205,206).

At CH1, the fundamental barrier for the recognition of sepsis was that clinical signs and symptoms in neonates are nonspecific. Therefore, when a neonate has as least one of sepsis-attributable risk factors, we should implement a multidisciplinary clinical, microbiological, and laboratory investigation to confirm the sepsis. The median (IQR) of CRP was 23.9 (3.5–58.3) mg/L (68). Although CRP is not specific for sepsis, a high CRP value could be an important and early marker for detecting of neonatal sepsis in our practice (68). CRP in combination with other acute reactants, such as procalcitonin could improve the effectiveness of sepsis detection (68).

The data demonstrated that 140 cases were found to be CAS (26.7%) and 384 cases were found to be HAS (73.3%). Previous studies have found that the nosocomial sepsis is a substantial problem in many NICUs (9,12,45,46,49,61). HAS is mostly caused by preventable issues, including contaminated parenteral nutrition preparation, intravenous infusion system, medical equipment, hospital environment, and the bad neonatal-maternal practices, particularly hand hygiene of care givers (9,12,45,46,49,61). Overall, the implementation of a multidimensional infection control strategy is required to reduce the significance of HAS in the neonatal population in our neonatal centre (9,12,45,46,49,61).

The introduction of antimicrobials in neonatal sepsis at CH1 were divided into two situations including empirical therapy (unknown pathogen) and blood culture-based therapy (known pathogen and its antimicrobial susceptibility profile). Empirical antimicrobial therapy was started immediately when an infectious condition was suspected or proven. In our practice, ampicillin plus gentamicin was the most commonly used therapy. We determined whether this treatment was appropriate by comparing the susceptibility of the pathogen isolated from the blood culture with the empirical therapy. We found high rates of prescription of apparently inappropriate antimicrobials. However, found no significant difference of inappropriate antimicrobial use between patients that died and survived in the univariable analysis.

The mortality of neonatal sepsis during the period of study was 13.2% (69/524 cases). The mortality of neonatal sepsis has been reported to be up to 36.9% (24/65 cases) in Cambodia (29) or 46% (49/106 cases) in Vietnam (27). Neonates, especially preterms, are at higher risk of sepsis and adverse outcomes associated with sepsis-induced inflammation (207). Extremely low birth weight (<1,000 g) as a mortality-associated factor in sepsis was consistent with previous findings in the literature. Sepsis was reported as a major cause of death in very low birthweight new-born infants with incidences of 25% in EOS and 18% in LOS (17,193,208). A study conducted in 1988 suggested that low birth weight (<2,500 g), increased the mortality of early onset neonatal group B streptococcal sepsis in neonates (209). A four-year historic cohort follow-up with an overall mortality rate of 9.5 % in southeastern Mexico found that low birth weight was associated with mortality in new-borns with sepsis (194). Low birth weight was also a significant risk factor for mortality in a study of neonatal sepsis in Turkey between 2010 and 2011 (195). Given the high mortality of sepsis in extremely low birth weight neonates,

efforts to reduce the incidence and increase the effectiveness of treatment in this vulnerable population are important strategies in clinical care.

Sclerema is described as a hardening of skin and subcutaneous adipose tissue in several cases of severe sepsis or with the presence of septic shock (196,210). Many case reports indicated that severe neonatal sepsis can present with sclerema (196,210). Here, sclerema was significantly associated with sepsis mortality in neonates. The outcome of sepsis with sclerema was almost always fatal even with the use of antimicrobials and intensive care. A randomised trial in 1997 of septic neonates with sclerema to undertake exchange transfusion reported a lower mortality rate of 50% (10 out of 20) in the study group, compared to 95% (19 out of 20) in the controls (p=0.003) (211).

Low white blood cell count is associated with an increased risk of infection (212,213). Our study found that white blood cell count <4,000/mm^3 significantly increased the risk of death due to sepsis in neonates. A study on the impact of white blood cell counts on septic patient outcome also indicated that white blood cell count <4,000/mm^3 in severe sepsis patients leads to more severe outcome than leucocytosis in severe sepsis patients (197). Another study of white blood cell count and its effect on mortality rate in neonatal sepsis suggested those with white blood cell count <5,000/mm^3 suffer the highest mortality rate, followed by cases with white blood cell count >30,000/mm^3 as compared with normal white blood cell count (198). A cohort study carried out among 460 neonates with sepsis in the Netherlands between 2006 and 2015 showed that thrombocytopenia in neonatal sepsis increases the risk of mortality nearly four-fold (199). This result concurs well with our finding that thrombocytopenia was an important laboratory result in neonatal sepsis and was among the most valuable factors for sepsis-associated mortality.

Globally, there have been limited data regarding factors that are associated with mortality in neonates. Our results generally share a number of similarities with the findings of another study in which thrombocytopenia were factors independently associated with mortality due to neonatal sepsis in a study of 424 very low birthweight neonates with LOS (200).

The limitations to this clinical study included the lack of an accepted consensus ensuring transferable practice, and the continuous change of diagnosis and treatment guidelines. Although this study was performed at the largest referral and tertiary children's hospital in Southern Vietnam, data collection at a single site may limit the applicability to other hospitals across the region. The clinical course of sepsis with negative blood culture, the interaction between sepsis and other infectious conditions, and the effect of antimicrobial prescription before hospitalization could not be comprehensively investigated.

In term of practical application, factors associated with mortality including sclerema, leukopenia <4,000/mm^3, thrombocytopenia <100,000/mm^3, base excess < –20 mEq/L, serum lactate >4 mmol/L, and hyperglycaemia >180 mg/dL will be clinically used as a risk-based approach in our neonatal centre to identify those at risk of poor outcome. Neonates with these features should be closely monitored to prevent further deterioration in the clinical course of sepsis that may lead to mortality.

4.4 Conclusion

Here, I identified key patient and clinical characteristics frequently observed in neonatal sepsis in our setting. These data will enable early risk stratification and interventions appropriate to LMIC settings, in addition to guiding the training and education of healthcare professionals in developing more of a risk-based approach to patient care including the importance of infection prevention and control measures. Factors associated with mortality including sclerema, leukopenia <4,000/mm^3, thrombocytopenia <100,000/mm^3, base excess < –20 mEq/L, serum lactate >4 mmol/L, and hyperglycaemia >180 mg/dL could be adopted as the standard reference to identity those at risk of poor outcome.

5 The microbiology of neonatal sepsis at Children's Hospital 1 in Ho Chi Minh City in Vietnam

5.1 Introduction

There is a remarkable heterogeneity regarding the definition of sepsis in neonates. The presence of a positive blood culture is the conventional "gold standard" for the diagnosis in a patient having clinical manifestations of neonatal sepsis (79–81). MDR pathogens account for approximately 30% of all global neonatal sepsis mortality that were attributable to AMR (7). Currently, AMR obviously poses a genuine problem to the management of sepsis in neonates, particularly infections caused by MDR Gram-negative bacteria, with the reality that the infection cannot be treated by many available antimicrobials (97,214,215). For example, the mortality of sepsis caused by MRSA in a prospective longitudinal study of sepsis in 17 neonatal centres in Australia was 24.6%, in comparison with 9.9% of that by MSSA (126).

Concerns regarding the AMR in neonatal sepsis have recently been invigorated in LMICs (97,216). A web-based survey by the NeoAMR (Neonatal Antimicrobial Resistance) network of 39 neonatal units from 12 LMICs highlighted that the prevalence of cephalosporin resistance among Gram-negative bacteria ranged from 26% to 84%, carbapenems resistance varied between 0% and 81%, and glycopeptide resistance in Gram-positive bacteria ranged from 0% to 45% (97). A cohort study in India (Delhi Neonatal Infection Study) observed a high proportion of MDR in *Acinetobacter* spp. (82%), *Klebsiella* spp. (54%), and *Escherichia coli* (38%). Methicillin resistance was recognised in 61% isolates of CoNS and 38% of *Staphylococcus aureus* isolates (216).

In SEA, AMR in cases of neonatal sepsis are being increasingly reported (56,58). However, there has been only limited published internationally regarding the bacteria and their AMR profiles causing neonatal sepsis in Vietnam. A 1-year study in a neonatal unit in Central Vietnam found that the most common causes of EOS were CoNS and *Staphylococcus aureus*, while of LOS were caused most commonly by *Acinetobacter*, CoNS and *Klebsiella pneumoniae* (27); MDR was found to be common among Gram-negative bacteria. In southern Vietnam, of 399 isolates from 385 neonates, the most frequently isolated organisms were *Klebsiella* spp. (n=78), *Acinetobacter* spp. (n=58), and *Escherichia coli* (n=21) (26). Mortality was found to be higher in neonatal sepsis with Gram-negative pathogens compared with Gram-positive organisms (26).

Understanding the microbiology of sepsis in hospitalized neonates is crucial in the development of rational management and treatment guidelines, especially in high-risk area for AMR in LMICs like Vietnam. Here, I performed a prospective observational study at CH1 in HCMC in Vietnam from January 2017 to June 2018 to characterise the bacterial causes of neonatal sepsis, to identify their AMR profile, and to investigate their potential association with mortality.

5.2 Results

5.2.1 Bacteria isolated from the blood culture of neonates with sepsis

Over the 18-month study period, from January 2017 to June 2018, there were 8,497 neonates admitted to CH1; 524 patients were recruited into my clinical study (Chapter 4). In total, 3,091 blood cultures were taken from the 524 neonatal sepsis patients; 469 bacterial isolates were generated (blood cultures positivity rate = 15.2%). Contaminants were recorded in 64 blood cultures (blood cultures contamination rate = 2.1%). No blood cultures grew >1 pathogen, resulting in 405 bacterial pathogens isolated from 344 (65.6%) culture-confirmed sepsis patients.

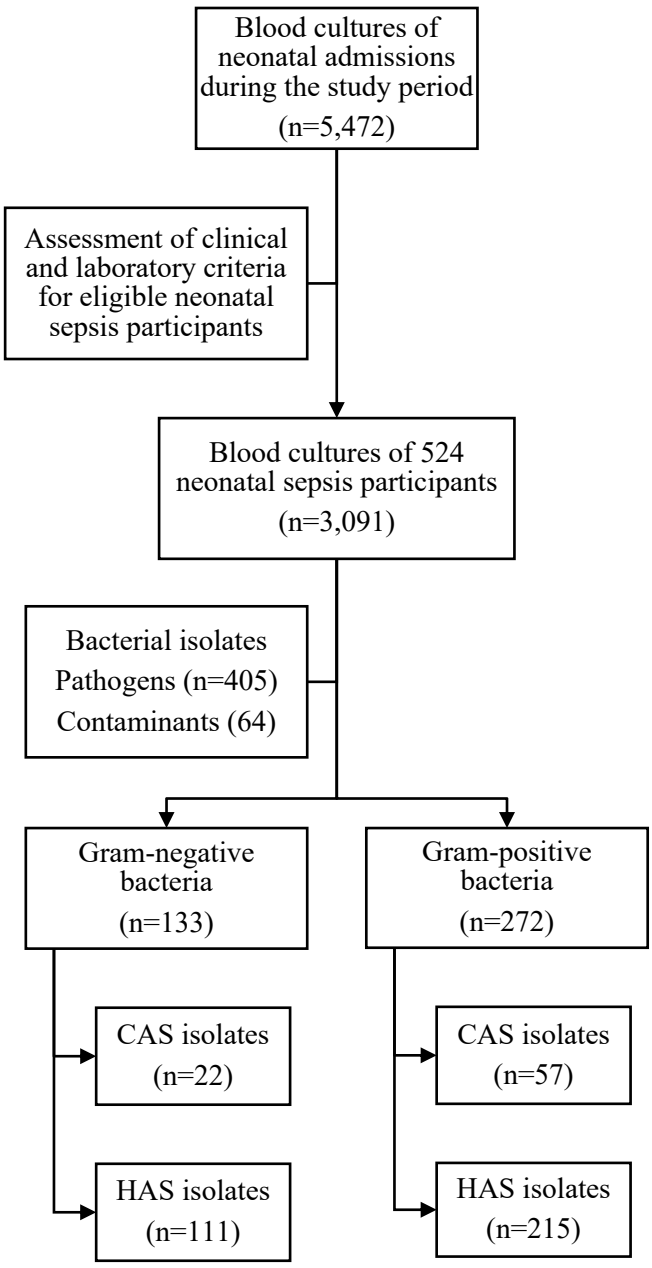

Figure 5.1 Flow chart for the microbiology study of neonatal sepsis at CH1

A total of 5,472 blood cultures were conducted in patients admitted to CH1 during the study period. Among 524 neonates recruited into the study of neonatal sepsis, 3,901 blood cultures were performed. There were 405 bacterial pathogens isolated from neonates with sepsis of which 133 isolates were Gram-negative bacteria and 272 isolates were Gram-positive bacteria. There were 111 Gram-negative bacteria and 215 Gram-positive bacteria isolated from neonates with HAS.

A time course of the positive blood samples is shown in Figure 5.1. The time series showed the stochastic variations in the number of positive blood cultures over the study period.

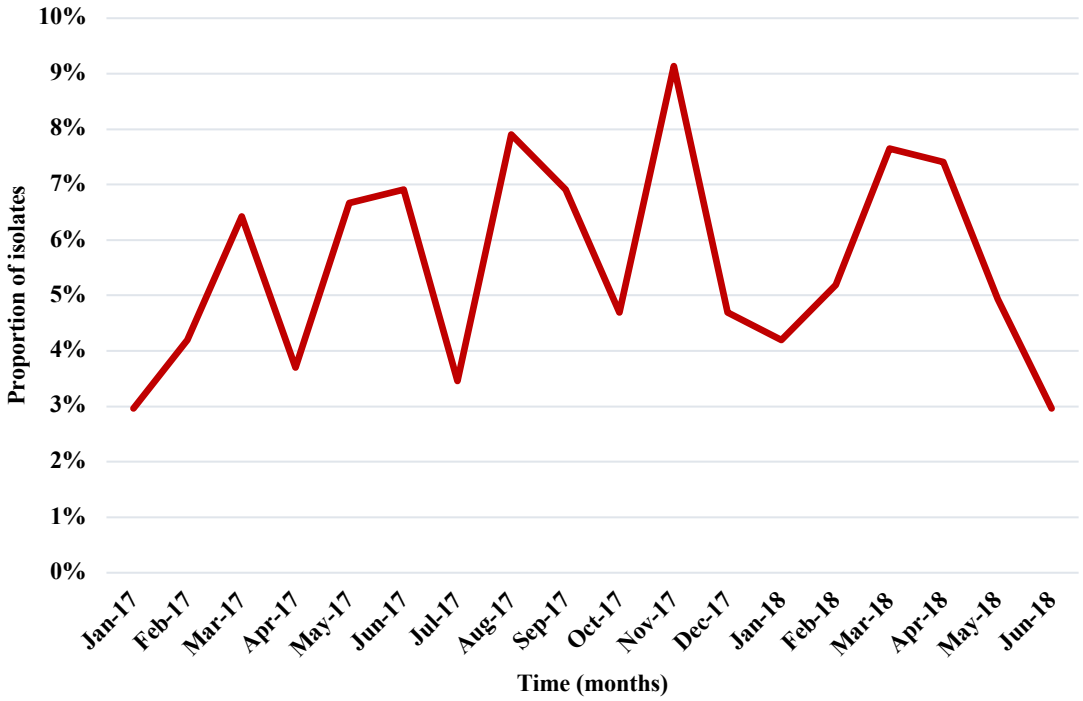

Figure 5.2 The distribution of bacterial isolates per month over the study period

Monthly isolation patterns from January 2017 to June 2018 represented by the red line showed the stochastic variations in the proportion of positive blood cultures obtained from neonates with sepsis. The proportion of bacterial isolates was at the peak in November 2017.

The 405 bacteria were comprised of 133 (32.8%) Gram-negative bacteria and 272 (67.2%). The most common Gram-negative pathogens were *Klebsiella* spp. (28/405, 6.9%), *Escherichia coli* (27/405, 6.7%), *Acinetobacter* spp. (16/405, 4.0%), and *Enterobacter* spp. (14/405, 3.5%). The majority of isolated Gram-positive bacteria were CoNS (232/405, 57.3%), *Staphylococcus aureus* (18/405, 4.4%), and *Streptococcus* spp. (10/405, 2.5%). Notably, I found no isolates of *Listeria monocytogenes* and only 2 isolates of GBS (2/405, 0.5%). The time trends with number of these major pathogens per month over the course of the study is shown in Figure 5.2. The full profile of Gram-negative and Gram-positive organisms are listed in Table 5.1.

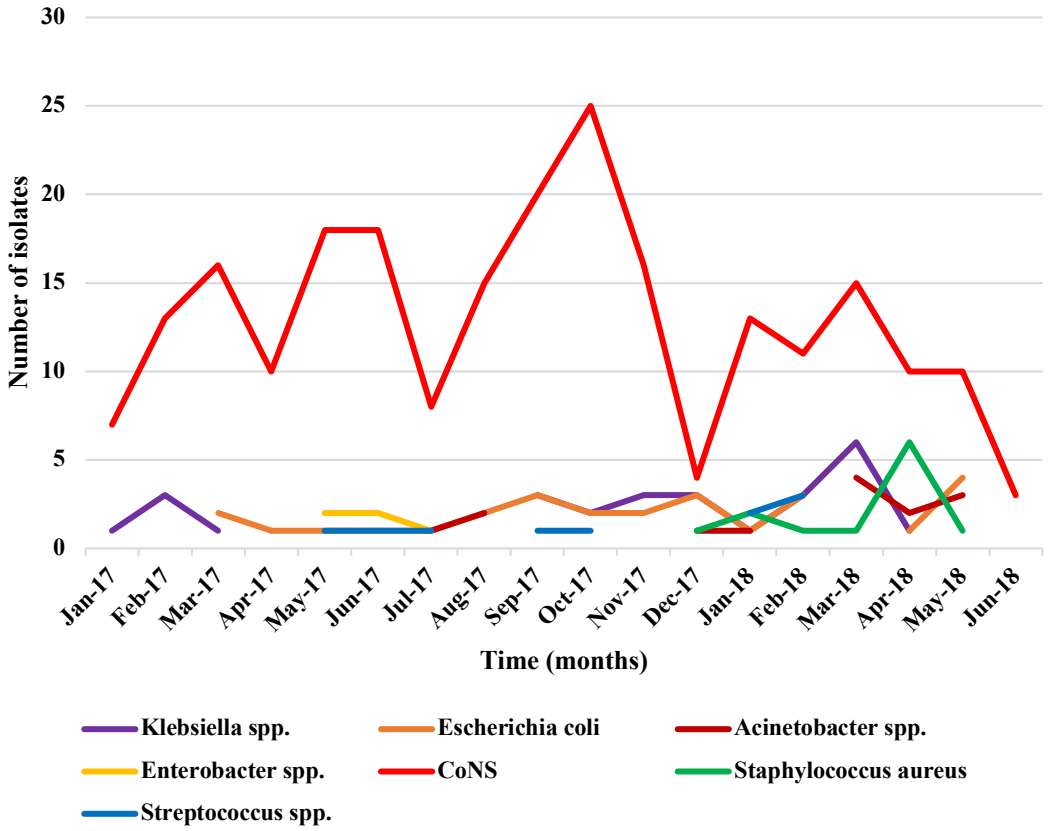

Figure 5.3 The number of major bacterial species per month over the study period

The number of *Klebsiella* spp. (purple), *Escherichia coli* (orange), *Acinetobacter* spp. (deep red), *Enterobacter* spp. (yellow), CoNS (light red), *Staphylococcus aureus* (green), and *Streptococcus* spp. (blue) per month from January 2017 to June 2018 represented by the colour lines showed the stochastic variations in the proportion of these isolated bacteria during the study period. The presence of *Staphylococcus aureus* (green) was only observed from December 2017 to May 2018. *Enterobacter* spp. (yellow) was only detected between May 2017 and July 2017. CoNS (red) reached the peak of its number in October 2017.

Table 5.1 The 405 bacteria isolated from blood culture of neonates with sepsis

Gram-negative bacteria	**133 (32.8%)**
Escherichia coli	27 (6.7%)
Klebsiella pneumoniae	27 (6.7%)
Acinetobacter baumannii	15 (3.7%)
Enterobacter cloacae	11 (2.7%)
Elizabethkingia meningoseptica	6 (1.5%)
Pandoraea sputorum	5 (1.2%)
Stenotrophomonas maltophilia	5 (1.2%)
Salmonella species	4 (1.0%)
Burkholderia cepacia	3 (0.7%)
Achromobacter xylosoxidans	3 (0.7%)
Pseudomonas aeruginosa	3 (0.7%)
Pantoea species	3 (0.7%)
Serratia marcescens	3 (0.7%)
Sphingomonas paucimobilis	2 (0.5%)
Proteus vulgaris	2 (0.5%)
Acinetobacter schindleri	1 (0.2%)
Cronobacter sakazaki	1 (0.2%)
Cupriavidus pauculus	1 (0.2%)
Enterobacter asburiae	1 (0.2%)
Enterobacter gergoviae	1 (0.2%)
Enterobacter kobei	1 (0.2%)
Halomonas aquamarina	1 (0.2%)
Klebsiella variicola	1 (0.2%)
Ochrobactrum intermedium	1 (0.2%)
Paenibacillus urinalis	1 (0.2%)
Pseudomonas fluorescens	1 (0.2%)
Pseudomonas stutzeri	1 (0.2%)
Ralstonia pickettii	1 (0.2%)
Roseomonas gilardii	1 (0.2%)

Table 5.1 Profile of 405 bacteria isolated from blood culture of neonates with sepsis (continued)

Gram-positive bacteria	**272 (67.2%)**
Staphylococcus aureus	18 (4.4%)
Micrococcus luteus	4 (1.0%)
Streptococcus mitis	4 (1.0%)
Enterococcus faecalis	3 (0.7%)
Enterococcus faecium	3 (0.7%)
Streptococcus pyogenes	3 (0.7%)
Streptococcus agalactiae	2 (0.5%)
Streptococcus gallolyticus	1 (0.2%)
Staphylococcus epidermidis	134 (33.1%)
Staphylococcus haemolyticus	41 (10.1%)
Staphylococcus hominis	27 (6.7%)
Staphylococcus warneri	14 (3.5%)
Staphylococcus capitis	11 (2.7%)
Staphylococcus saprophyticus	2 (0.5%)
Staphylococcus kloosii	1 (0.2%)
Staphylococcus lugdunensis	1 (0.2%)
Staphylococcus pasteuri	1 (0.2%)
Rothia amarae	1 (0.2%)
Kytococcus schroeteri	1 (0.2%)

5.2.2 The antimicrobial susceptibility of Gram-negative bacteria isolated from blood cultures in neonatal sepsis patients at CH1

Overall, I found that *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., and *Enterobacter* spp. were the major Gram-negative bacteria isolated from neonatal sepsis at CH1 from January 2017 to June 2018. These Gram-negative organisms had a low prevalence of susceptibility against cephalosporins (ceftriaxone, cefotaxime, ceftazidime, cefepime), and fluoroquinolones (pefloxacin, ciprofloxacin, levofloxacin), all of which were commonly used at CH1. The Gram-negative organisms that exhibited the highest prevalence of resistance were *Acinetobacter baumannii*. The antimicrobial susceptibility profiles of Gram-negative bacteria isolated from blood culture of neonates with sepsis at CH1 are shown in Figure 5.3.

Theoretically, the most effective antimicrobials against *Klebsiella* spp. were imipenem (21/28; 75% susceptibility), meropenem (24/28; 85.7% susceptibility), and colistin (28/2; 100% susceptibility). However, *Klebsiella* spp. isolates showed no susceptibility to ampicillin (0/28; 0.0%), cefotaxime (0/28; 0.0%), ceftazidime (0/28; 0.0%), ticarcillin-clavulanic acid (0/28; 0.0%), and pefloxacin (0/28; 0%); there was low susceptibility against ceftriaxone (1/28; 3.6%), cefepime (1/28; 3.6%), and ciprofloxacin (1/28; 3.6%).

Imipenem (20/27; 74.1% susceptible), meropenem (24/27; 88.9% susceptible), and colistin (27/27; 100.0% susceptible) were theoretically the most effective antimicrobial agents against *Escherichia coli*. The potentially least effective antimicrobials against *Escherichia coli* infections were ampicillin (1/27; 3.7% susceptible), ticarcillin-clavulanic acid (2/27; 7.4% susceptible), ceftriaxone (3/27; 11.1% susceptible), cefotaxime (3/27;

11.1% susceptible), ceftazidime (3/27; 11.1% susceptible), cefepime (4/27; 14.8% susceptible), and pefloxacin (3/27; 11.1% susceptible).

Notably, *Acinetobacter* spp. exhibited low susceptibility to nearly all antimicrobials, even to carbapenems, including both imipenem (2/16; 12.5% susceptible) and meropenem (2/16; 12.5% susceptible). Furthermore, only 1/16 (6.3%) of *Acinetobacter* spp. isolates was susceptible to ceftriaxone, and only 2/16 (12.5%) of *Acinetobacter* spp. isolates were susceptible to ticarcillin-clavulanic acid, ceftazidime, ciprofloxacin, and levofloxacin. We observed that only trimethoprim-sulfamethoxazole (11/16; 68.8% susceptible) and colistin (16/16; 100% susceptible) could be potentially used to effectively treat sepsis associated with *Acinetobacter* spp.

Enterobacter spp. exhibited broad susceptibility to levofloxacin (12/14; 85.7% susceptible), meropenem (12/14; 85.7% susceptible), imipenem (13/14; 92.9% susceptible), and colistin (13/14; 92.9% susceptible). Conversely, susceptibility against ampicillin (0/14; 0.0% susceptible), ceftriaxone (0/14; 0.0% susceptible), and cefotaxime (2/14; 14.3% susceptible) in *Enterobacter* spp. were low. Lastly, 1/14 (7.1%) of *Enterobacter* spp. isolates were non-susceptible to colistin.

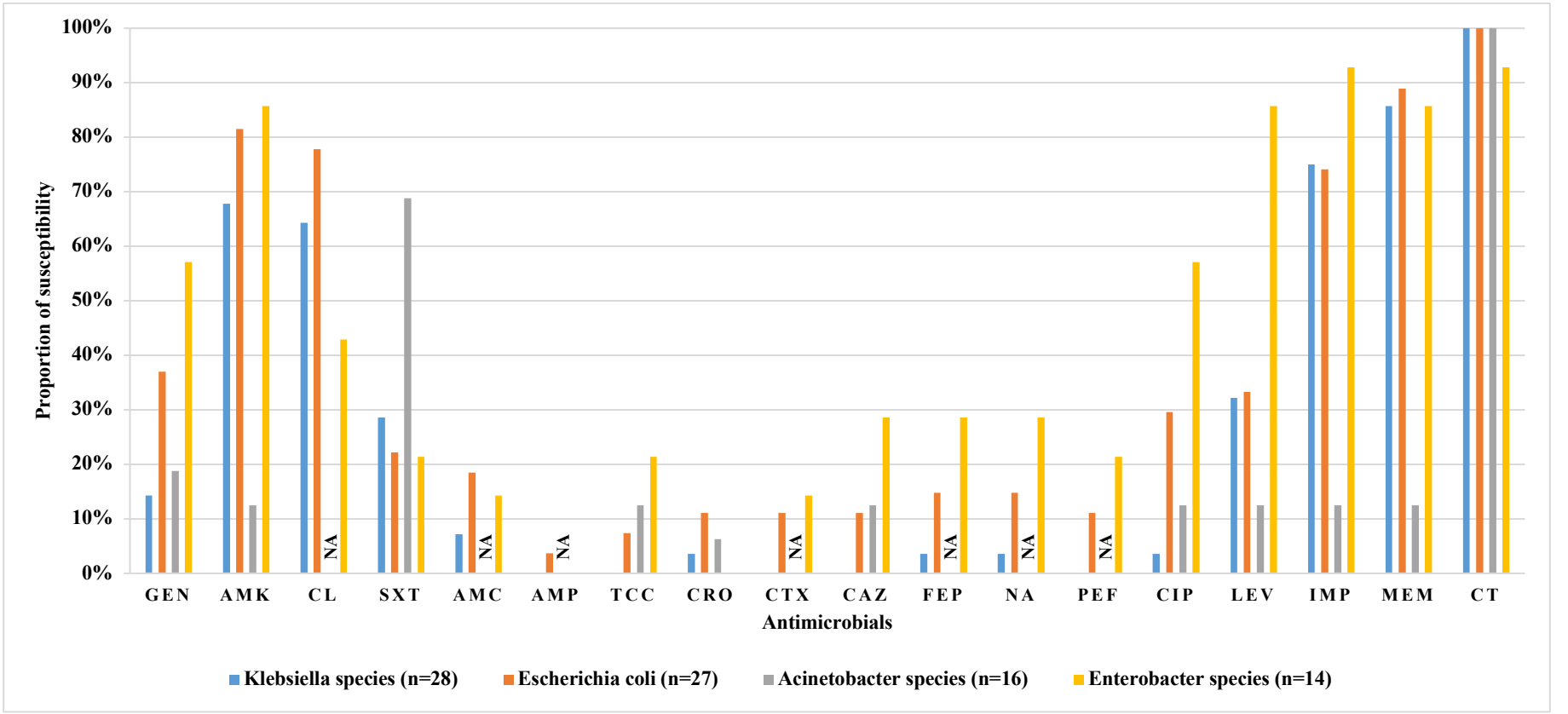

Figure 5.4 Antimicrobial susceptibility of major Gram-negative bacteria isolated from blood culture in neonatal sepsis at CH1

The antimicrobial susceptibility proportions of major Gram-negative bacteria including *Klebsiella* spp. (blue), *Escherichia coli* (orange), *Acinetobacter* spp. (grey), and *Enterobacter* spp. (yellow) were illustrated in four columns correponding to each antimicrobial. The susceptibility of these bacteria to cephalosporins and fluoroquinolones were generally low. *Acinetobacter* spp. (grey) showed low susceptibility to the majority of antimicrobials including imipenem and meropenem and only clolistin and trimethoprim-sulfamethoxazole were found to be effective against this pathogen. GEN: gentamicin, AMK: amikacin, CL: chloramphenicol, SXT: trimethoprim-sulfamethoxazole, AMC: amoxicillin-clavulanic acid, AMP: ampicillin, TCC: ticarcillin-clavulanic acid, CRO: ceftriaxone, CTX: cefotaxime, CAZ: ceftazidime, FEP: cefepime, NA: nalidixic acid, PEF: pefloxacin, CIP: ciprofloxacin, LEV: levofloxacin, IMP: imipenem, MEM: meropenem, CT: colistin, NA: not available.

5.2.3 The antimicrobial susceptibility of Gram-positive bacteria isolated from blood cultures in neonatal sepsis at CH1

The susceptibility profiles of Gram-positive bacteria isolated from blood culture of neonates with sepsis at CH1 are shown in Figure 5.4. The most common Gram-positive organisms isolated from the blood of neonates were CoNS. CoNS had limited susceptibility to penicillin (11/232; 4.7% susceptible). Notably, only 23/232 isolates (9.9%) of CoNS were susceptible to oxacillin. All CoNS (232/232) were susceptible to vancomycin and the susceptibility of CoNS to linezolid was 97.8% (227/232 susceptible).

Staphylococcus aureus had limited susceptibility to penicillin, ciprofloxacin, and clindamycin; only 1/18 (5.6%) of isolates were susceptible to penicillin and 3/18 (16.7%) of isolates were susceptible to ciprofloxacin and clindamycin. Only 5/18 (27.8%) *Staphylococcus aureus* isolates were susceptible to oxacillin, indicating that the proportion of MRSA infections was 72.2% (13/18 isolates). However, all of the *Staphylococcus aureus* isolates were susceptible to vancomycin (18/18). The prevalence of linezolid susceptibility in *Staphylococcus aureus* was 17/18 (94.4%; susceptible). All of the isolated *Streptococcus* spp. were resistant to erythromycin (0/10) and they additionally exhibited low susceptibility to tetracycline (1/10; 10.0% susceptible), penicillin (4/10; 40.0% susceptible), levofloxacin (4/10; 40.0% susceptible), and clindamycin (5/10; 50.0% susceptible). All *Streptococcus* spp. (10/10 isolates) were susceptible to vancomycin and linezolid.

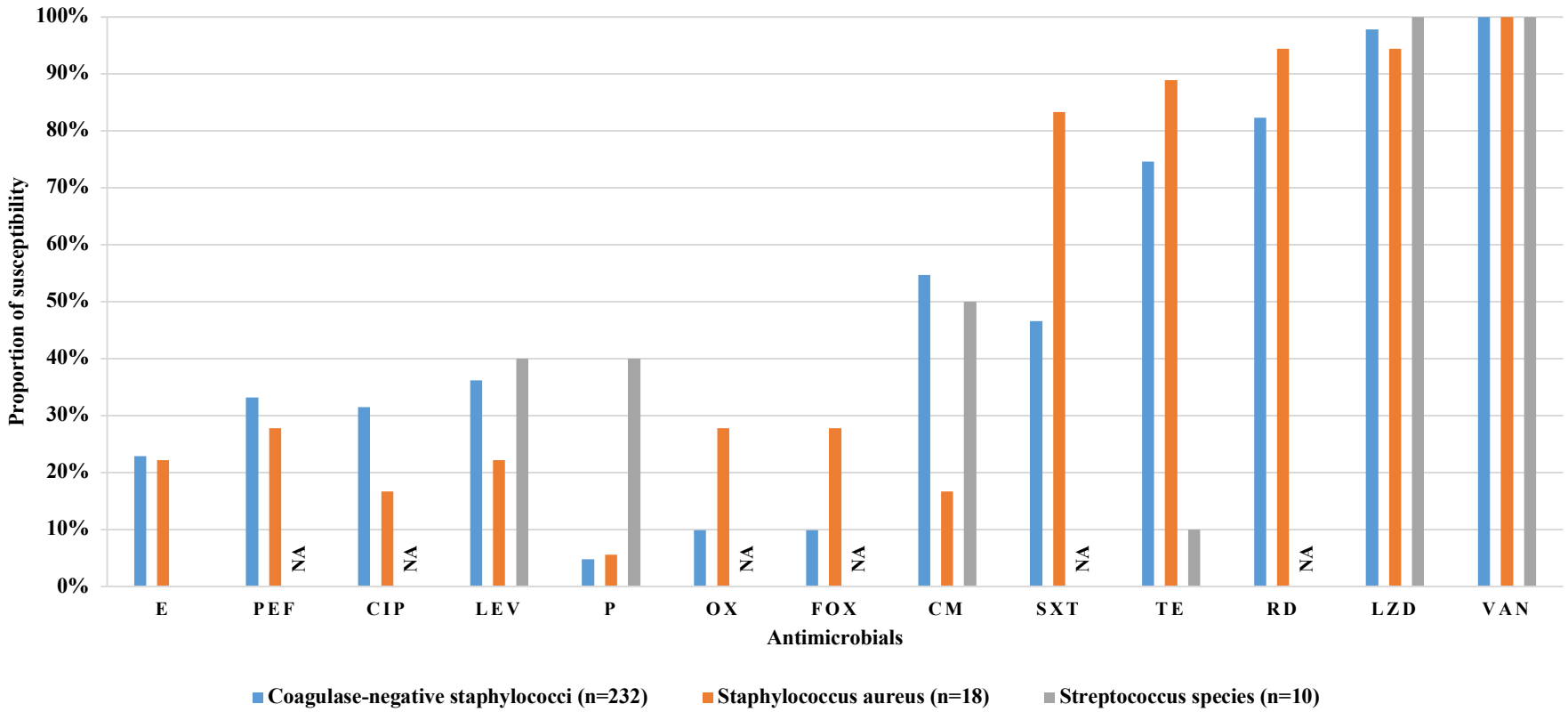

Figure 5.5 The antimicrobial susceptibility of major Gram-positive bacteria isolated from blood culture in neonatal sepsis at CH1
The antimicrobial susceptibility proportions of major Gram-positive bacteria including CoNS (blue), *Staphylococcus aureus* (orange), and *Streptococcus* spp. (grey) were illustrated in three columns correponding to each antimicrobial. The susceptible of CoNS (blue) and *Staphylococcus aureus* (orange) to oxacillin were low but these major Gram-positive bacteria were all susceptible to vancomycin. E: erythromycin, PEF: pefloxacin, CIP: ciprofloxacin, LEV: levofloxacin, P: penicillin, OX: oxacillin, FOX: cefoxitin, CM: clindamycin, SXT: trimethoprim-sulfamethoxazole, TE: tetracycline, RD: rifampicin, LZD: linezolid, VAN: vancomycin, NA: not available.

5.2.4 Major bacteria isolated from CAS and HAS at CH1

I investigated the key organisms associated with CAS and HAS to determine any potential difference in the profile of bacterial these two important classifications of sepsis (Table 5.2). I found that the isolation of *Klebsiella* spp. (p=0.003) and *Staphylococcus aureus* (p=0.006) were found to be significantly associated with CAS and HAS, respectively, in neonatal infections (Table 5.2).

Table 5.2 Bacteria associated with CAS and HAS at CH1

	CAS isolates (n=79)	**HAS isolates (n=326)**	***P*-values***
***Klebsiella* spp.**	0 (0.0)	28 (8.6)	**0.003**
Escherichia coli	8 (10.1)	19 (5.8)	0.169
Acinetobacter spp.	3 (3.8)	13 (4.0)	0.937
Enterobacter spp.	2 (2.5)	12 (3.7)	0.615
CoNS	42 (53.2)	190 (58.3)	0.409
Staphylococcus aureus	8 (10.1)	10 (3.1)	**0.006**
Streptococcus spp.	3 (3.8)	7 (2.1)	0.396

Values are n (%), *Chi-squared test or Fisher's exact test.

5.2.5 Clinical features of neonates with sepsis caused by major Gram-negative bacteria

The key clinical features of neonates with sepsis caused by the major Gram-negative bacteria at CH1 are summarized in Table 5.3. It can be observed that all sepsis cases caused by *Klebsiella* spp. (23/23 patients) and *Enterobacter* spp. (14/14 patients) were LOS (Table 5.3). However, there was no significant difference in the onset of sepsis caused by *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., and *Enterobacter* spp. using logistic regression analysis (p=0.409) (Table 5.3). Additionally, all cases of sepsis caused by *Klebsiella* spp. (23/23 patients) and 85.7% (12/14 patients) of sepsis caused by *Enterobacter* spp. were HAS (Table 5.3). However, there were no significant difference in the acquisition of sepsis caused by *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., and *Enterobacter* spp. using logistic regression analysis (p=0.658) (Table 5.3).

An NTISS severity score of >10 was identified in 23/23 patients (100.0%) with sepsis caused by *Klebsiella* spp. However, a NTISS severity score >10 was not significantly associated with infection caused by *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., and *Enterobacter* spp. using logistic regression (p=0.682) (Table 5.3). However, severe sepsis was found to significantly different between four groups of patients with sepsis caused by *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., and *Enterobacter* spp. using logistic regression (p<0.001) (Table 5.3). Notably, both *Acinetobacter* spp. (OR=6.1; 95% CI, 1.9–19.1; p=0.002) and *Enterobacter* spp. (OR=4.0; 95% CI, 1.4–11.8; p=0.011) were found to be associated with severe sepsis (Table 5.3).

The distribution of septic shock was significantly different between four groups of patients with sepsis caused by *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., and

Enterobacter spp. using logistic regression (p<0.001) (Table 5.3). *Klebsiella* spp. (OR=3.2; 95% CI, 1.2–8.2; p=0.017), *Escherichia coli* (OR=3.7; 95% CI, 1.4–9.5; p=0.007), and *Acinetobacter* spp. (OR=6.2; 95% CI, 2.0–19.5; p=0.002) were all significantly associated with septic shock (Table 5.3).

Staying in hospital >21 days was determined in 20/23 patients (87.0%) with sepsis caused by *Klebsiella* spp. and there was a significant association between the length of hospital stay >21 days and sepsis caused by *Klebsiella* spp. (OR=5.8; 95% CI, 1.7–19.8; p=0.005) with logistic regression analysis. (Table 5.3). In addition, a hospital length of stay of >21 days was significantly different between the four groups of patients with sepsis caused by *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., and *Enterobacter* spp. using logistic regression (p=0.023) (Table 5.3).

Using logistic regression, I found a significant difference in the mortality between four groups of patients with sepsis caused by *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., and *Enterobacter* spp. (p=0.039) (Table 5.3). Notably, *Acinetobacter* spp. was found to significantly associated with the mortality of neonates with sepsis (OR=4.8; 95% CI, 1.5–15.1; p=0.008) (Table 5.3).

Table 5.3 Clinical features of neonates with sepsis caused by major Gram-negative bacteria at CH1

	***Klebsiella* spp. sepsis patients (n=23)**	***Escherichia coli* sepsis patients (n=22)**	***Acinetobacter* spp. sepsis patients (n=13)**	***Enterobacter* spp. sepsis patients (n=14)**	***P*-values***
Onset of sepsis					0.409
EOS	0 (0.0)	4 (18.2)	1 (7.7)	0 (0.0)	
LOS	23 (100.0) –	18 (81.8) OR=0.4; 95% CI, 0.1–1.4; *p*=0.150	12 (92.3) OR=1.1; 95% CI, 0.1–8.5; *p*=0.952	14 (100.0) –	
Acquisition of sepsis					0.658
CAS	0 (0.0)	6 (27.3)	3 (23.1)	2 (14.3)	
HAS	23 (100.0) –	16 (72.7) OR=1.1; 95% CI, 0.4–2.8; *p*=0.903	10 (76.9) OR=1.2; 95% CI, 0.3–4.5; *p*=0.792	12 (85.7) OR=2.4; 95% CI, 0.5–10.8; *p*=0.259	
Severity					
NTISS >10	23 (100.0) –	16 (72.7) OR=0.9; 95% CI, 0.3–2.3; *p*=0.824	11 (84.6) OR=1.7; 95% CI, 0.4–7.8; *p*=0.506	12 (85.7) OR=2.0; 95% CI, 0.4–9.2; *p*=0.363	0.682
Severe sepsis	9 (39.1) OR=2.4; 95% CI, 1.0–5.8; *p*=0.050	8 (36.4) OR=2.3; 95% CI, 0.9–5.7; *p*=0.068	8 (61.5) **OR=6.1; 95% CI, 1.9–19.1; *p*=0.002**	7 (50.0) **OR=4.0; 95% CI, 1.4–11.8; *p*=0.011**	**<0.001**
Septic shock	7 (30.4) **OR=3.2; 95% CI, 1.2–8.2; *p*=0.017**	7 (31.8) **OR=3.7; 95% CI, 1.4–9.5; *p*=0.007**	6 (46.2) **OR=6.2; 95% CI, 2.0–19.5; *p*=0.002**	3 (21.4) OR=2.2; 95% CI, 0.6–8.0; *p*=0.248	**<0.001**
Outcomes					
Duration of stay >21 days	20 (87.0) **OR=5.8; 95% CI, 1.7–19.8; *p*=0.005**	12 (54.5) OR=1.0; 95% CI, 0.4–2.5; *p*=0.932	7 (53.8) OR=0.9; 95% CI, 0.3–2.8; *p*=0.865	8 (57.1) OR=1.2; 95% CI, 0.4–3.4; *p*=0.794	**0.023**
Mortality	3 (13.0) OR=1.0; 95% CI, 0.3–3.6; *p*=0.965	5 (22.7) OR=2.2; 95% CI, 0.8–6.3; *p*=0.126	5 (38.5) **OR=4.8; 95%CI, 1.5–15.1; *p*=0.008**	4 (28.6) OR=3.1; 95% CI, 0.9–10.1; *p*=0.067	**0.039**

Values are n (%) or median (IQR), *Logistic regression.

5.2.6 Clinical features of neonates with sepsis caused by major Gram-positive bacteria

The key clinical characteristics of neonates with sepsis caused by major Gram-positive bacteria at CH1 are summarized in Table 5.4. It can be seen that all cases of sepsis caused by *Staphylococcus aureus* (17/17 patients), and 97.1% of cases caused by CoNS (204/210 patients) were LOS (Table 5.4). The onset of sepsis caused by CoNS, *Staphylococcus aureus*, and *Streptococcus* spp. were found to be significant different using logistic regression analysis (p<0.001) (Table 5.4). There was a significant association between sepsis caused by CoNS and LOS (OR=5.0; 95% CI, 2.1–12.1; p<0.001) (Table 5.4).

Notably, 81.0% of sepsis caused by CoNS (170/210 patients) were HAS and there was a significant association between sepsis caused by CoNS and HAS (OR=2.0; 95% CI, 1.3–3.0; p=0.002) (Table 5.4). In addition, there was a significant difference in the acquisition of sepsis caused by CoNS, *Staphylococcus aureus*, and *Streptococcus* spp. by logistic regression analysis (p=0.004) (Table 5.4).

There was significant association between the severity scoring system NTISS >10 and sepsis caused by CoNS (OR=1.8; 95% CI, 1.2–2.8; p=0.007) (Table 5.4). In addition, the severity scoring system NTISS >10 was significantly different between three groups of patients with sepsis caused by CoNS, *Staphylococcus aureus*, and *Streptococcus* spp. using logistic regression (p=0.025) (Table 5.4). Additionally, there was a significant association between sepsis caused by CoNS and a hospital stay >21 days (OR=1.8; 95% CI, 1.2–2.5; p=0.002) with logistic regression analysis. (Table 5.4). A hospital stay >21 days was significantly different between three groups of patients with sepsis caused by CoNS, *Staphylococcus aureus*, and *Streptococcus* spp. using logistic regression (p=0.006)

(Table 5.4). There were no significant differences in terms of severe sepsis (p=0.874), septic shock (p=0.607), and mortality (p=0.927) between three groups of patients with sepsis caused by CoNS, *Staphylococcus aureus*, and *Streptococcus* spp. using logistic regression (Table 5.4).

Table 5.4 Clinical features of neonates with sepsis caused by major Gram-positive bacteria at CH1

	CoNS sepsis patients (n=210)	***Staphylococcus aureus* sepsis patients (n=17)**	***Streptococcus* spp. sepsis patients (n=10)**	***P*-values***
Onset of sepsis				**<0.001**
EOS	6 (2.9)	0 (0.0)	1 (10.0)	
LOS	204 (97.1) **OR=5.0; 95% CI, 2.1–12.1; *p*<0.001**	17 (100.0) –	9 (90.0) OR=1.4; 95% CI, 0.2–11.0; *p*=0.778	
Acquisition of sepsis				**0.004**
CAS	40 (19.0)	8 (47.1)	3 (30.0)	
HAS	170 (81.0) **OR=2.0; 95% CI, 1.3–3.0; *p*=0.002**	9 (52.9) OR=0.4; 95% CI, 0.2–1.2; *p*=0.097	7 (70.0) OR=1.1; 95% CI, 0.3–4.2; *p*=0.943	
Severity				
NTISS >10	174 (82.9) **OR=1.8; 95% CI, 1.2–2.8; *p*=0.007**	12 (70.6) OR=0.8; 95% CI, 0.3–2.3; *p*=0.680	6 (60.0) OR=0.6; 95% CI, 0.2–2.1; *p*=0.389	**0.025**
Severe sepsis	47 (22.4) OR=1.0; 95% CI, 0.6–1.5; *p*=0.828	5 (29.4) OR=1.4; 95% CI, 0.5–4.1; *p*=0.533	3 (30.0) OR=1.4; 95% CI, 0.4–5.7; *p*=0.612	0.874
Septic shock	31 (14.8) OR=1.2; 95% CI, 0.7–1.9; *p*=0.550	4 (23.5) OR=2.0; 95% CI, 0.6–6.4; *p*=0.229	2 (20.0) OR=1.7; 95% CI, 0.4–8.4; *p*=0.505	0.607
Outcomes				
Duration of stay >21 days	134 (63.8) **OR=1.8; 95% CI, 1.2–2.5; *p*=0.002**	9 (52.9) OR=1.0; 95% CI, 0.4–2.6; *p*=0.979	3 (30.0) OR=0.4; 95% CI, 0.1–1.7; *p*=0.226	**0.006**
Mortality	30 (14.3) OR=1.2; 95% CI, 0.7–1.9; *p*=0.564	2 (11.8) OR=0.9; 95% CI, 0.2–4.0; *p*=0.885	1 (10.0) OR=0.8; 95% CI, 0.1–6.3; *p*=0.811	0.927

Values are n (%) or median (IQR), *Logistic regression.

5.2.7 Mortality of sepsis patients and bacterial identification

The mortality of sepsis patients, bacterial identification, and related features including the acquisition of sepsis (HAS or CAS) and time of sepsis (EOS or LOS) are shown in Table 5.3 and Table 5.4. I compared the bacterial identifications from patients who died and survived using Chi-squared test to identify any association between specific bacteria and the mortality of patients. The proportions of *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., *Acinetobacter baumannii*, *Enterobacter* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus* spp. were compared between the patients that died or survived (Table 5.4). *Acinetobacter* spp. (p=0.006) and specifically *Acinetobacter baumannii* (p=0.003) was found to be associated with mortality (Table 5.5).

Table 5.5 Mortality of sepsis patients and bacterial identification

Patients with sepsis caused by	Died (n=69)	Survived (n=455)	*P*-values*
Klebsiella spp.	3 (4.3)	20 (4.4)	0.986
Escherichia coli	5 (7.2)	17 (3.7)	0.176
***Acinetobacter* spp.**	5 (7.2)	8 (1.8)	**0.006**
Acinetobacter baumannii	5 (7.2)	7 (1.5)	**0.003**
Enterobacter spp.	4 (5.8)	10 (2.2)	0.084
CoNS	30 (43.5)	180 (39.6)	0.536
Staphylococcus aureus	2 (2.9)	15 (3.3)	0.862
Streptococcus spp.	1 (1.4)	9 (2.0)	0.765

Values are n (%), *Chi-squared test.

5.2.8 Non-susceptibility of *Acinetobacter baumannii* and the mortality

I performed further investigation of AMR of 15 isolates of *Acinetobacter baumannii*, defining MDR as resistance to >3 classes of any tested antimicrobials. Extensive drug resistance (XDR) was defined as resistance to all antimicrobials except colistin (Figure 5.6). In total, 14/15 (93.3%) isolates of *Acinetobacter baumannii* were MDR and 3/15 (20.0%) isolates of *Acinetobacter baumannii* were XDR. *Acinetobacter baumannii* was the most antimicrobial resistant Gram-negative bacteria in this study. Using Chi-squared and Fisher's exact tests, I compared the non-susceptibility of *Acinetobacter baumannii* isolates to different antimicrobials between those that died and those that survived (Table 5.6). There was no significant association between the antimicrobial susceptibility of *Acinetobacter baumannii* isolates and mortality (Table 5.6).

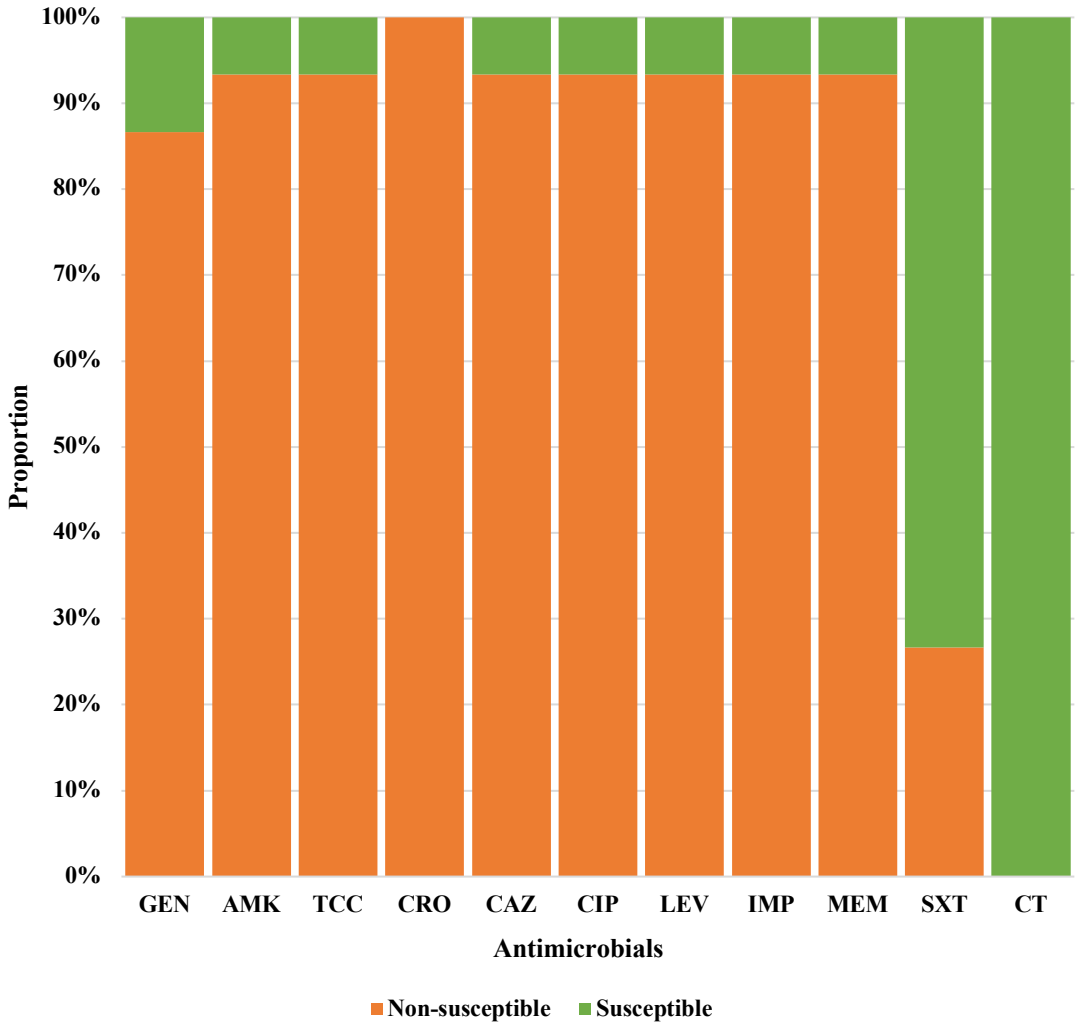

Figure 5.6 The antimicrobial susceptibility profile of 15 isolates of *Acinetobacter baumannii* isolated from blood culture in neonatal sepsis at CH1

The proportions of antimicrobial non-susceptibility (orange) and susceptibility (green) of 15 isolates of *Acinetobacter baumannii* were illustrated. *Acinetobacter baumannii* showed low susceptibility (green) to the majority of antimicrobials including imipenem and meropenem. It can be seen that only clolistin and trimethoprim-sulfamethoxazole were found to be effective against this pathogen. GEN: gentamicin, AMK: amikacin, TCC: ticarcillin-clavulanic acid, CRO: ceftriaxone, CAZ: ceftazidime, CIP: ciprofloxacin, LEV: levofloxacin, IMP: imipenem, MEM: meropenem, SXT: trimethoprim-sulfamethoxazole, CT: colistin.

Table 5.6 Non-susceptibility of *Acinetobacter baumannii* and the mortality of neonates with sepsis at CH1

Non-susceptibility of *Acinetobacter baumannii isolates*	Died (n=8)	Survived (n=7)	*P*-values*
Gentamicin	8 (100.0)	5 (71.4)	0.200
Amikacin	8 (100.0)	6 (85.7)	0.467
Ticarcillin-clavulanic acid	8 (100.0)	6 (85.7)	0.467
Ceftriaxone	8 (100.0)	7 (100.0)	–
Ceftazidime	8 (100.0)	6 (85.7)	0.467
Ciprofloxacin	8 (100.0)	6 (85.7)	0.467
Levofloxacin	8 (100.0)	6 (85.7)	0.467
Imipenem	8 (100.0)	6 (85.7)	0.467
Meropenem	8 (100.0)	6 (85.7)	0.467
Trimethoprim-sulfamethoxazole	1 (12.5)	3 (42.9)	0.185
Colistin	0 (0.0)	0 (0.0)	–
MDR	8 (100.0)	6 (85.7)	0.467
XDR	1 (12.5)	2 (28.6)	0.438

Values are n (%), *Chi-squared test or Fisher's exact test.

5.2.9 Non-susceptibility of CoNS and the mortality of neonates with sepsis

For further investigation of the AMR of 232 isolates of CoNS, I defined the MDR of CoNS as resistance to >3 classes of any tested antimicrobials (Figure 5.7). The XDR of CoNS was defined as resistance to all antimicrobials except vancomycin and linezolid. Notably, 205/232 isolates of CoNS (88.4%) were MDR, and 6/232 isolates of CoNS (2.6%) were XDR. Using Chi-squared and Fisher's exact tests, I compared the non-susceptibility of CoNS isolates to different antimicrobials between those that died and those that survived (Table 5.7). Non-susceptibility of CoNS isolates to erythromycin (p=0.024) and rifampicin (p=0.011) were significantly associated with death (Table 5.7).

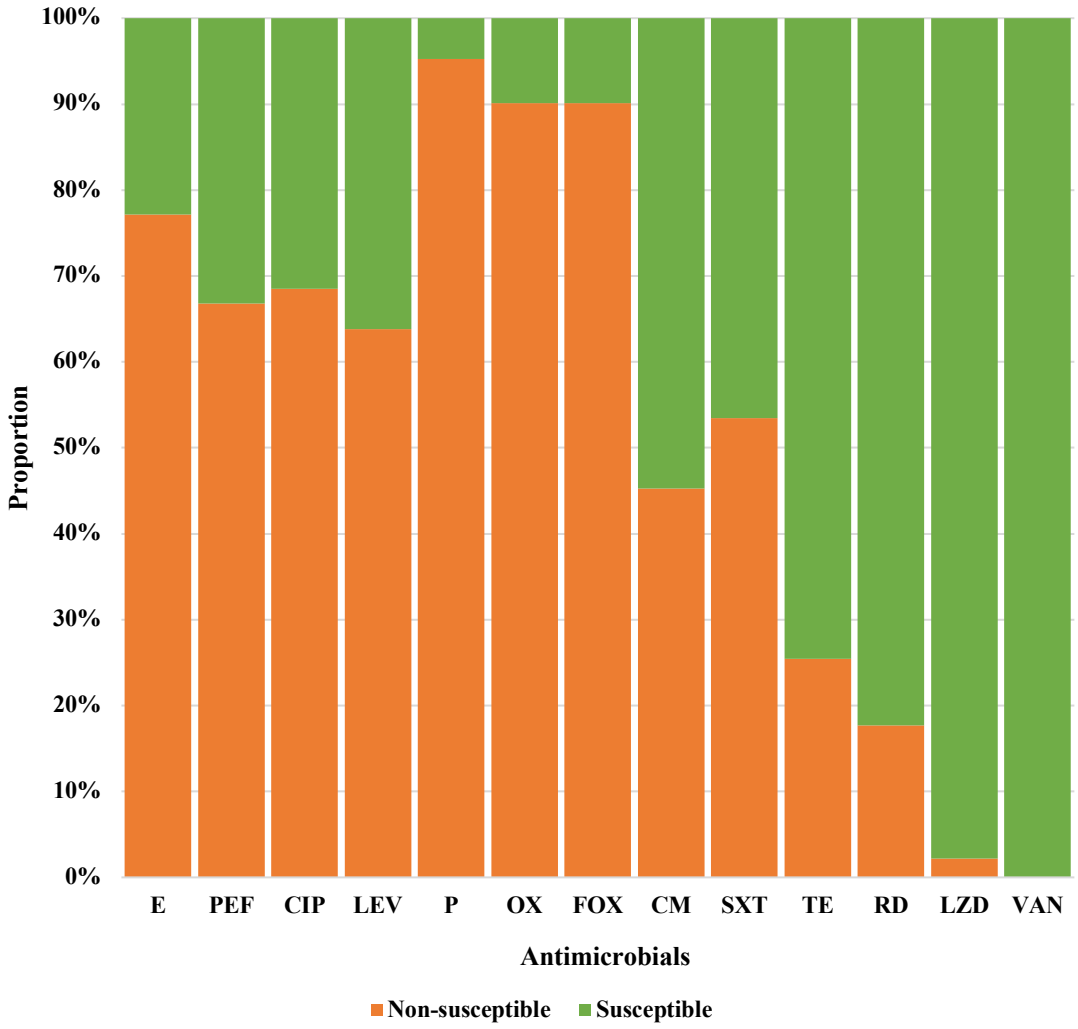

Figure 5.7 The antimicrobial susceptibility of 232 isolates of CoNS isolated from blood culture in neonatal sepsis at CH1

The proportions of antimicrobial non-susceptibility (orange) and susceptibility (green) of 232 isolates of CoNS were illustrated. The susceptibility of CoNS (green) to oxacillin were low but the susceptibility of this bacteria to vancomycin was 100%. E: erythromycin, PEF: pefloxacin, CIP: ciprofloxacin, LEV: levofloxacin, P: penicillin, OX: oxacillin, FOX: cefoxitin, CM: clindamycin, SXT: trimethoprim-sulfamethoxazole, TE: tetracycline, RD: rifampicin, LZD: linezolid, VAN: vancomycin.

Table 5.7 Non-susceptibility of CoNS and the neonatal sepsis mortality at CH1

Non-susceptibility of CoNS isolates	Died (n=36)	Survived (n=196)	*P*-values*
Erythromycin	33 (91.7)	146 (74.5)	**0.024**
Pefloxacin	22 (61.1)	133 (67.9)	0.429
Ciprofloxacin	24 (66.7)	135 (68.9)	0.793
Levofloxacin	24 (66.7)	124 (63.3)	0.696
Penicillin	32 (88.9)	189 (96.4)	0.050
Oxacillin	33 (91.7)	176 (89.8)	0.730
Cefoxitin	33 (91.7)	176 (89.8)	0.730
Clindamycin	15 (41.7)	90 (45.9)	1.000
Trimethoprim-sulfamethoxazole	19 (52.8)	105 (53.6)	0.930
Tetracycline	8 (22.2)	51 (26.0)	0.631
Rifampicin	1 (2.8)	40 (20.4)	**0.011**
Linezolid	0 (0.0)	5 (2.6)	1.000
Vancomycin	0 (0.0)	0 (0.0)	–
MDR	32 (88.9)	173 (88.3)	0.915
XDR	0 (0.0)	6 (3.1)	0.594

Values are n (%), *Chi-squared test or Fisher's exact test.

5.2.10 The use of empirical antimicrobials in neonatal sepsis

At CH1, first line of empirical antimicrobials includes ampicillin, cefotaxime, and gentamicin. The second line antimicrobials are ceftazidime, cefepime, ciprofloxacin, levofloxacin, ticarcillin, co-trimoxazole, amikacin, oxacillin, and clindamycin. The "last resort" antimicrobials are imipenem, meropenem, and colistin for Gram-negative bacteria and linezolid and vancomycin for Gram-positive bacteria. Empirical antimicrobial therapy was initiated immediately when sepsis was suspected in all cases as per the current hospital guidelines. I defined whether the empirical antimicrobial therapy was appropriate by comparing the treatment with the susceptibility of the pathogen isolated from the blood culture. A pathogen was isolated from blood culture in 344/524 (65.6%) patients; 261/344 (75.9%) patients were given antimicrobials.

Empirical antimicrobials, categorised by first line, second line, and third line and the susceptibility of major bacteria isolated from the blood culture in neonatal sepsis at CH1 are shown in Table 5.8. Overall, the Gram-negative pathogens *Klebsiella* spp., *Escherichia coli*, and *Enterobacter* spp. showed low susceptibility to first line and second line antimicrobials. The MDR and XDR *Acinetobacter baumannii* could only be treated by colistin. For Gram-positive bacteria, *Staphylococcus aureus* was common, but all of these organisms were susceptible to vancomycin.

Table 5.8 Antimicrobials and susceptibility of major isolated bacteria in neonatal sepsis at CH1

	***Klebsiella* spp.** (n=28, %)	***E. coli*** (n=27, %)	***A. baumannii*** (n=15, %)	***Enterobacter* spp.** (n=14, %)	**CoNS** (n=232, %)	***S. aureus*** (n=18, %)	***Streptococcus* spp.** (n=10, %)
First line							
Ampicillin	0 (0.0)	1 (3.7)		0 (0.0)			
Cefotaxime	0 (0.0)	3 (11.1)		2 (14.3)			
Gentamicin	4 (14.3%)	10 (37.0)	2 (13.3)	8 (57.1)			
Second line							
Ceftazidime	0 (0.0)	3 (11.1)	1 (6.7)	4 (28.6)			
Cefepime	1 (3.6)	4 (14.8)		4 (28.6)			
Ciprofloxacin	1 (3.6)	8 (29.6)	1 (6.7)	8 (57.1)	73 (31.5)	3 (16.7)	
Levofloxacin	9 (32.1)	9 (33.3)	1 (6.7)	12 (85.7)	84 (36.2)	4 (22.2)	4 (40.0)
Ticarcillin	0 (0.0)	2 (7.4)	1 (6.7)	3 (21.4)			
Co-trimoxazole	8 (28.6)	6 (22.2)	11 (73.3)	3 (21.4)	108 (46.6)	15 (83.3)	
Amikacin	19 (67.9)	22 (81.5)	1 (6.7)	12 (85.7)			
Oxacillin					23 (9.9)	5 (27.8)	
Clindamycin					127 (54.7)	3 (16.7)	5 (50.0)
Third line							
Imipenem	21 (75.0)	20 (74.1)	1 (6.7)	13 (92.9)			
Meropenem	24 (85.7)	24 (88.9)	1 (6.7)	12 (85.7)			
Colistin	28 (100.0)	27 (100.0)	15 (100.0)	13 (92.9)			
Linezolid					227 (97.8)	17 (94.4)	10 (100.0)
Vancomycin					232 (100.0)	18 (100.0)	10 (100.0)

5.3 Discussion

Here, I described the bacterial profile and investigated the antimicrobial susceptibility patterns of the 7 major bacterial species (*Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., *Enterobacter* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus* spp.) associated with neonatal sepsis at CH1. The bacterial profile of neonatal sepsis at CH1 were relatively consistent with the results of previous studies in other countries as these bacterial pathogens are commonly found in the NICU settings (9,12,45,46,49,61). Formerly, research in central Vietnam also found that *Acinetobacter* spp., *Klebsiella pneumoniae*, CoNS and *Staphylococcus aureus* were common organisms isolated from neonatal sepsis patients (27).

Vertical and maternal transmission of pathogens during delivery, as a result of chorioamnionitis and bacterial colonisations in the urinary or gastrointestinal tract of the mother, is often associated with EOS in new-born infants (9,11–14). However, the number of *Listeria monocytogenes* and GBS at CH1 was limited in comparison to those in high income countries, where they were common pathogens of EOS in neonates (22,46). The explanations for the lack of these organisms could be the microbiological capacity was inadequate to detect these organisms, or an absence of these bacteria related to the prehospital use of antimicrobials or death. Notably, CH1 does not provide obstetric or intrapartum care, therefore, very little information on maternal carriage of *Listeria monocytogenes* and GBS were available; it was also possible that the rates of carriage and infection of these organisms are low in LMICs in Asia (217).

CoNS constituted 57.3% (232/405) of all isolated pathogens of neonatal sepsis at CH1 during the study period. The contaminant-pathogen decision of CoNS was made based on

the clinical relevance, independent assessments and discussions of two qualified medical microbiologists until reaching a final conclusion. In our practice at CH1, prematurity, low birth weight, central venous catheterisation, abnormal haematology, parenteral nutrition, and lipid infusion are factors that clinically indicated the sepsis relevance of CoNS (218–220).

I observed that most of the commonly used antimicrobials were not effective *in vitro* against Gram-negative sepsis at CH1. Generally, this study found that Gram-negative bacteria showed low susceptibility to various antimicrobials including ampicillin, ticarcillin, 3rd generation cephalosporins (cefotaxime, ceftazidime), 4th generation cephalosporin (cefepime), fluoroquinolones (ciprofloxacin, levofloxacin), and aminoglycosides (gentamicin, amikacin). Therefore, empirical antimicrobial therapy without carbapenems and colistin probably failed to treat the sepsis quickly in neonates at CH1.

Alarmingly, the susceptibility rates Gram-negative bacteria against carbapenems were low. Particularly, *Acinetobacter baumannii* showed resistance to all first line and second line antimicrobials used at CH1 and were resistant to imipenem and meropenem (only 1/15, 6.7% of isolates were susceptible). It could be seen that only colistin (100.0% or 15/15 of isolates were susceptible) and co-trimoxazole (11/15, 73.3% of isolates were sensitive) were effectively *in vitro* against *Acinetobacter baumannii* at CH1.

Methicillin resistance was also an important issue for Gram-positive bacteria when; 23/232 (9.9%) of CoNS isolates and 5/18 (27.8%) of *Staphylococcus aureus* isolates were susceptible to oxacillin. This is particularly worrying, given that neonatal sepsis is a

complex and rapidly progressing syndrome and empirical antimicrobial therapy is the only treatment option at CH1 according to our local practice guidelines. Fortunately, major Gram-positive bacteria including CoNS, *Staphylococcus aureus*, and *Streptococcus* spp. were all susceptible to vancomycin.

I did not find an association between the non-susceptibility of isolated pathogens and the outcomes of patients. I compared antimicrobial non-susceptibility isolated organisms between those that died and survived sepsis. I found a potential association between *Acinetobacter* spp. and *Acinetobacter baumannii* with neonatal sepsis mortality. However, there was no significant association between the non-susceptibility of *Acinetobacter* spp. and the mortality of sepsis patients in my study. Only the non-susceptibility of CoNS isolates to erythromycin and rifampicin were significantly different between the dead and survival groups; however, these two antimicrobials are not commonly used for the treatment of sepsis in neonates in this setting.

5.4 Conclusion

Here, I observed that *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., *Enterobacter* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus* spp. were the most common bacteria causing sepsis in neonates at CH1. These infections we commonly associated low antimicrobial susceptibility rates, thus posing a further threat in this dangerous condition. Given the susceptibility of isolated pathogens, our choices of empirical antimicrobial therapy including cephalosporins, quinolones, oxacillin and aminoglycosides were not effective *in vitro* against many Gram-negative and Gram-positive bacteria. The MDR and XDR profiles of *Acinetobacter baumannii* were common, indicating that infections caused by this pathogen could only be treated with colistin, and trimethoprim-

sulfamethoxazole. *Acinetobacter baumannii* was significantly associated with mortality of patients however, an association between antimicrobial non-susceptibility and neonatal sepsis mortality was not observed. Therapy with non-carbapenem antimicrobials provided limited coverage for many bacterial pathogens of neonatal sepsis at CH1 during the study period.

6 The genomics of *Acinetobacter baumannii* associated with neonatal sepsis at Children's Hospital 1 in Ho Chi Minh City in Vietnam

6.1 Introduction

Neonatal sepsis caused by *Acinetobacter baumannii* can occur sporadically in NICUs with risk factors including low birth weight, total parenteral nutrition, and with indwelling central venous catheterisation (221). *Acinetobacter baumannii* is an aerobic, pleomorphic, and nonmotile Gram-negative bacillus, and an opportunistic nosocomial pathogen (222). This human pathogen is responsible for a variety of infections, of which ventilator-associated pneumonia (VAP) and BSIs are the most common; mortality rates of infections associated with this organism can reach 35% (223). MDR *Acinetobacter baumannii* infection has been reported to lead to a higher mortality rate compared with infections caused by antimicrobial susceptible variants (224). In a study at a NICU in Indonesia, 24 *Acinetobacter baumannii* was isolated from 80 neonates, 7 isolates were MDR (225). In addition, a case-control study in a Thailand found that the mortality in those with a carbapenem-resistant *Acinetobacter baumannii* infection (n=14) was 42.9% (98).

Blood culture conventionally plays a pivotal role in identifying the bacteria causing neonatal sepsis and their antimicrobial susceptibility profiles (226). By utilizing genomics, researchers have further ability to investigate the molecular characteristics of pathogenic bacteria and to offer novel insights into the mechanisms triggering neonatal sepsis (227,228). The *Acinetobacter baumannii* population primarily responsible for hospital infections in humans largely consists of two globally distributed clones, GC1 and GC2 (229). Fluoroquinolone resistance in *Acinetobacter baumannii* is primarily mediated through non-synonymous mutations in *gyrA* and *parC*, which inhibit the drug binding to

the active site (230). Carbapenem resistance of *Acinetobacter baumannii* has also been associated with changes in the expression of penicillin-binding proteins (231) or the AdeABC efflux pump (232).

I found that MDR *Acinetobacter baumannii* was associated with sepsis in the hospitalised neonates. I considered that a dominant single clone of *Acinetobacter baumannii* had been introduced and then potentially maintained on the NICU and was associated with neonatal infections. Here, in a prospective observational study at CH1 in HCMC in Vietnam from January 2017 to June 2018, I used genomic approach to study the population of AMR *Acinetobacter baumannii*. I aimed to investigate the relationship of these organisms thus providing insights into the local epidemiology of this important pathogen.

6.2 Results

6.2.1 Clinical features of sepsis caused by *Acinetobacter baumannii*

During the study period of January 2017 to June 2018 I isolated 15 *Acinetobacter baumannii* from the blood cultures of 12 patients admitted to the NICU of CH1. The majority of these neonates were diagnosed with LOS (11/12, 91.7%) and the vast majority were associated with HAS (9/12, 75%).

The *Acinetobacter baumannii* cases were generally associated with severe sepsis, with 8/12 (66.7%) of cases having severe sepsis and 6/12 (50%) patients having septic shock. Overall, the median (IQR) NTISS score was 30 (27–41) and the vaginal/Caesarean delivery ratio was 1:1. In this small group of sepsis patients, like in many other cases, prematurity (7/12; 58.3%) and low birth weight (8/12; 66.7%) were common. Pneumonia was diagnosed in 7/12 patients (58.3%) and ventilator support was required in 10/12 patients (83.3%). The median (IQR) length of stay was 16 (6–55) days. Ultimately, neonatal sepsis caused by *Acinetobacter baumannii* had a mortality rate of 41.7% (5/12 patients). The clinical features of neonates with sepsis caused by *Acinetobacter baumannii* are shown in Table 6.1 and Figure 6.1.

Table 6.1 Clinical features of *Acinetobacter baumannii* sepsis patients

N = 12 patients	n (%) or median (IQR)
Onset of sepsis	
EOS	1 (8.3)
LOS	11 (91.7)
Acquisition of sepsis	
CAS	3 (25.0)
HAS	9 (75.0)
Severity	
NTISS severity scoring	30 (27–41)
Severe sepsis	8 (66.7)
Septic shock	6 (50.0)
Obstetrical features	
Vaginal delivery	6 (50.0)
Caesarean section delivery	6 (50.0)
Demographic features	
Preterm birth	7 (58.3)
Low birth weight	8 (66.7)
Significant features	
Pneumonia	7 (58.3)
Congenital heart diseases	5 (41.7)
Peritonitis	4 (33.3)
Ventilator support	10 (83.3)
Parenteral nutrition	10 (83.3)
Blood products transfusion	10 (83.3)
Invasive surgery/procedures	8 (66.7)
Outcomes	
Duration of stay (days)	16 (6–55)
Mortality	5 (41.7)

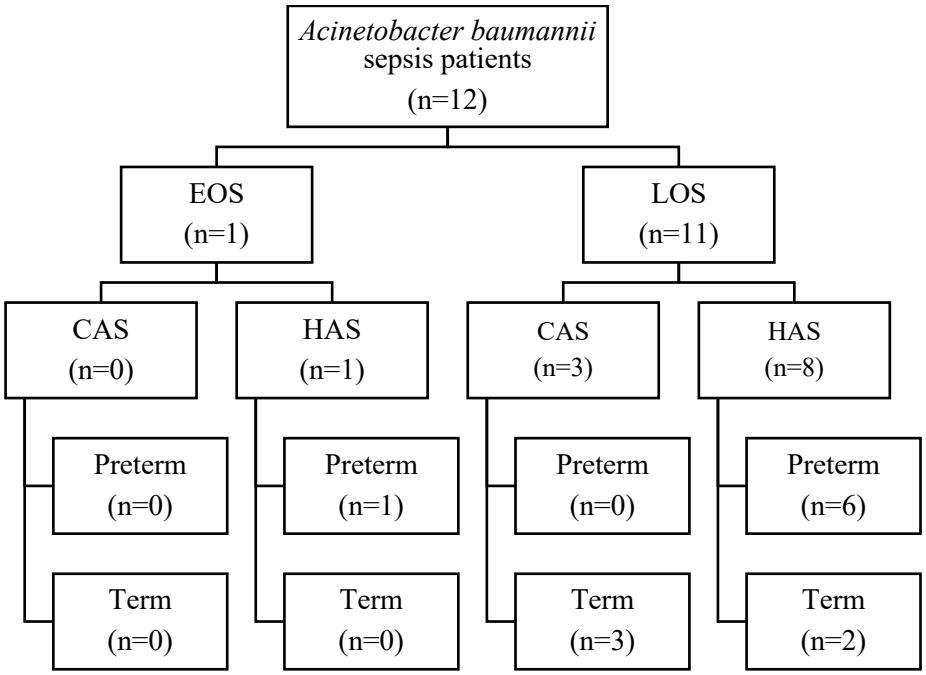

Figure 6.1 Clinical features of *Acinetobacter baumannii* sepsis neonates

There were 12 neonates with sepsis caused by *Acinetobacter baumannii*. Most of these cases were LOS (11/12 patients) with a majority of HAS (8/11 patients). Only 1 preterm neonate was diagnosed as EOS and HAS. All other 6 preterm neonates were found to have LOS and HAS. Among 5 term neonates with sepsis caused by *Acinetobacter baumannii*, 3 cases had CAS and 2 cases had HAS.

6.2.2 Genome sequencing of *Acinetobacter baumannii* isolates

The 15 *Acinetobacter baumannii* from the blood of neonates with sepsis were submitted for genome sequencing and the associated data were compiled. Mapping the sequences of these 15 isolates collected from these neonates to the genome of *Acinetobacter baumannii* A1656-2 showed that these new sequences were well aligned with the reference genome; resulting in a mapping average of 86.7%; the average of depth coverage was approximately 40X. Ultimately, using these data, a phylogenetic tree was constructed using a total 3,948 SNPs that were identified in the core genome from the mapping (Figure 6.2A).

After determining the MLST loci, we found that the majority (12/15; 80%) of isolates were sequence type ST570, which belongs within genomic complex 2 (GC2) (Figure 6.1B). The remainder 2/15 isolates (13.3%) were ST164 (Figure 6.1B). The sequence type of 1/15 isolate (6.7%) was not determined as this was a new sequence type that was not described in the MLST database of *Acinetobacter baumannii* (Figure 6.2B).

The metadata from the cases were overlaid next to the phylogeny to add additional context to the 15 isolates and revealed that 12/15 (80%) isolates were epidemiologically associated with HAS. The remaining 3/15 (20%) isolates were epidemiologically associated with CAS. Notably, 8/15 organisms were isolated from the blood of five different patients who all died, these organisms were all found to be the same ST (ST570) (Figure 6.2B).

According to the AMR profiles (i.e., assessing AMR gene content and relating it back to predicted phenotype) I found that all 12 of the ST570 isolates had predicted resistant

against amikacin, ciprofloxacin, ceftazidime, carbapenems (meropenem and imipenem), ticarcillin- clavulanic acid, levofloxacin and ceftriaxone. The same observation was true for the ST164 isolates. I additionally found that all the ST570 were resistant to gentamicin; however, only 1/3 of non-ST570 isolates had predicted resistance against this antimicrobial. Notably, a large proportion of the *Acinetobacter baumannii* isolates were predicted to be susceptible to co-trimoxazole and colistin, (11/15, 73% and 15/15, 100%, respectively).

To better understand the potential mechanisms of antimicrobial resistance to critical antimicrobials used in high dependency units, I next performed a screen of the sequence data to identify known resistance genes. This was performed by constructing a heat-map (Figure 6.2A) of the resulting compendium of resistance genes that were associated with the sepsis causing organisms. The rationale for this screen was not only to identify the key resistance genes, but also to observe if these genes were restricted to specific genotypes/STs.

This analysis revealed that genes conferring to resistance to beta-lactam antimicrobials, including bla_{MBL}, bla_{ADC}, and bla_{A2} had been acquired by the majority of the most of isolates. Additionally, all of isolates within lineage ST570 contained $bla_{\text{OXA-23}}$, which encodes resistance to carbapenems. The $bla_{\text{OXA-23}}$ gene was generally located in a gene cassette within an ISAba1 insertion sequence, which belongs to the IS4 transposase family and is commonly found within clinical isolates of *Acinetobacter baumannii*. This particular transposon carrying the $bla_{\text{OXA-23}}$ has been identified a common transposon and has the potential for horizontal transfer between organisms, consequently leading to widespread carbapenem resistance in *Acinetobacter baumannii* found in hospital

infections (233). Further, I found that genes conferring resistance to macrolides, namely *mphE* and *msrE*, were present in all of the ST570 isolates and 11/12 (91.6%) of these isolates additionally carried the *armA* gene, encoding resistance to aminoglycosides.

Aiming to understand additional clinical implications of these infections I plotted the duration of the various patients infected with *Acinetobacter baumannii* to investigate if there was any overlap between cases or to see if there was any association between duration of the case and a specific genotype. Notably, this plot showed that that patients who were infected with an ST570 stayed for a shorter time in hospital than other *Acinetobacter baumannii* cases; and this was commonly associated with death (Figure 6.2B). It is probable that the progression of the disease in these patients was rapid and may have been associated with a lack of a response to the antimicrobial therapy.

The median (IQR) of the length of stay of patients with ST570 isolates were 7 (4–25) days while of non-ST570 isolates were 107 (6–141) days but the Wilcoxon rank-sum test found no significant difference of the hospital stay between patients with ST570 isolates and patients with non-ST570 isolates (p=0.14). In addition, the Fisher's exact test found no significant difference in the mortality between ST570 isolates (8/12, 66.7%) and non-ST570 isolates (0/3, 0.0%) (p=0.08).

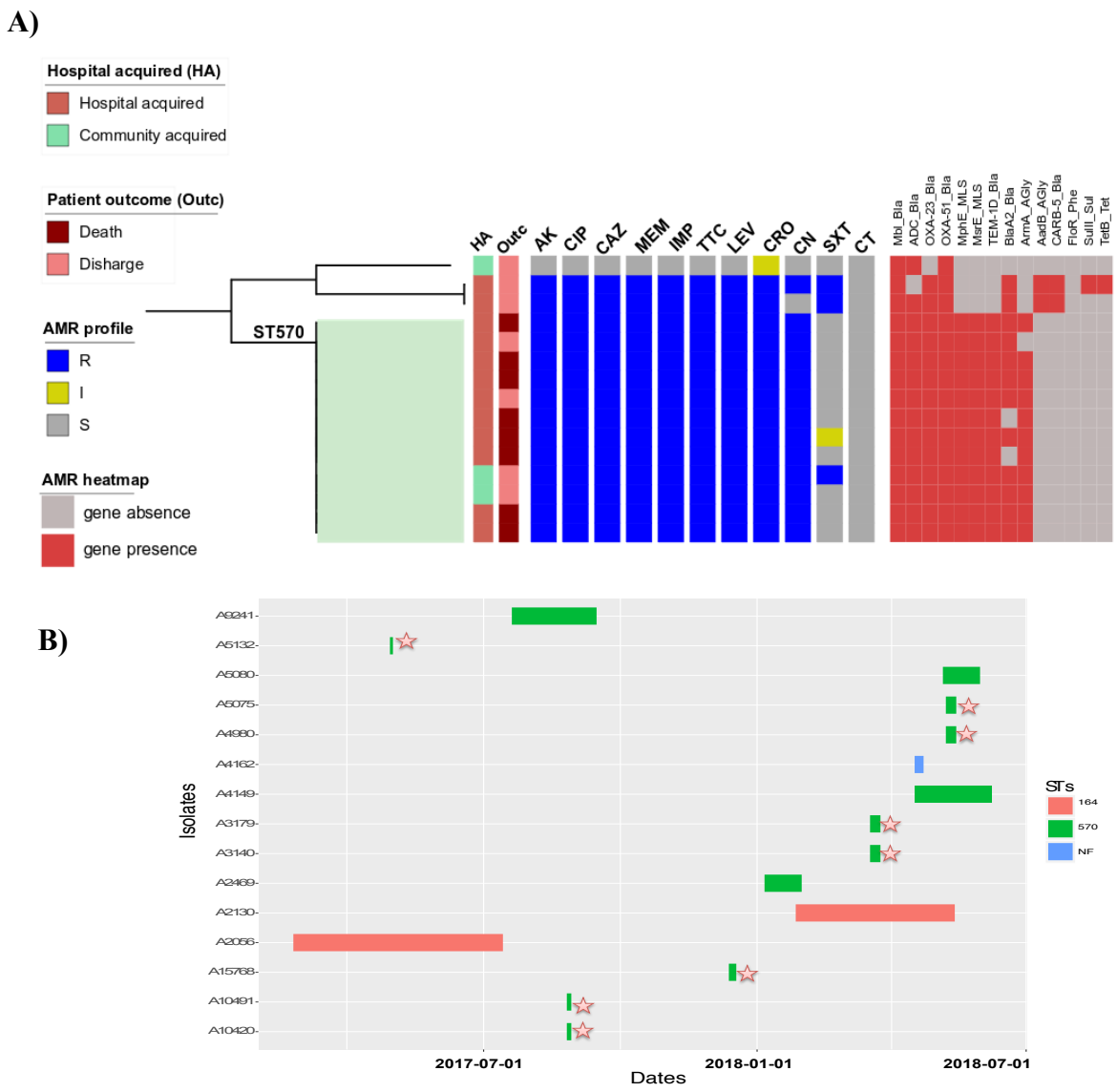

Figure 6.2 Genome sequencing of *Acinetobacter baumannii* isolates

A. Maximum likelihood (ML) tree constructed from 3948 core SNPs leaving from mapping 15 isolates to the Acinetobacter baumannii reference genome. From the phylogeny to the first column: the acquisition of sepsis (orange: hospital acquired infection; green: the community acquired infection); the second column: the patient outcomes (pink- discharged; dark brown- died); the next 10 columns: representation of the antimicrobial susceptibility profile (blue-resistance; yellow-intermediate; and grey-susceptibility) as done by Kirby-Bauer disc diffusion against AK (amikacin), CIP (ciprofloxacin), CAZ (ceftazidime), MEM (meropenem), IMP (imipenem), TTC (ticarcillin-clavulanic acid), LEV (levofloxacin), CRO (ceftriaxone), CN (gentamicin), SXT (trimethoprim/sulfamethoxazole), CT (colistin); the heatmap: resistome genes (red-gene presence; and grey-gene absence). **B.** Hospital duration of patients. Stars: patients died.

6.2.3 Inferring a timescale of a potential outbreak

The emergence of *Acinetobacter baumannii* ST570 as a major hospital pathogen has been described previously in a study originating from an adult ICU in a sentinel infectious disease hospital in Vietnam between 2010 to 2011. Here, due to the timing and the genetic conservation we suspected a further outbreak of this genotype during this study.

With the aim of investigating the genomic diversity and characteristics of these ST570 novel isolates from 2018, we included 16 isolates from previous study in the adult ICU in 2010/2011 and the 9 ST570 isolates from this study to reconstruct the maximum likelihood phylogenetic tree. The resulting tree showed that new isolates from CH1 were an extension of the same lineage with the organisms isolated previously. The long connecting branch suggested that this was a continuation of the same genotype that had been circulating in unrelated Vietnamese hospital some seven years previously.

To investigate whether the data was sufficient to identify a temporal signal between the genome sequences, I used a simple of linear regression method associating the sampling time with the root-to-tip divergence of the sequences and then estimated the slope coefficient. I identified a strong correlation between the sampling dates and the root-to-tip distances (R^2=0.9907) (Figure 6.3A), suggesting that this was indeed a continuation of the previous outbreak which had accumulated mutations at a regular rate in the intervening years.

Next, I randomly reassigned the sampling time of sequences 20 times and generated the mean rate estimates for these different data sets. Plotting the mean rates of the correct sampling dates and those of randomized- dated sets confirmed that there was no overlap

between the 95% credible interval between the original mean rate and the mean rates of the date randomizations, which resulted in a strong temporal signal that could be used for further analysis (Figure 6.3B).

Using these data, I performed a BEAST analysis to infer a timescale for all ST570 in the phylogenetic tree. This tree indicated that the mutation rate of ST570 was 1.91 x 10^{-6} substitution/site/year or 7.6 SNPs/genome/year (95% HPD 1.45 x 10^{-6} to 2.39 x 10^{-6} or 5.7–9.5 SNPs/genome/year). Using this mutation rate, I could estimate that the time to the most common ancestor was 9.2 years previously, in early 2009 [95% HPD, 2008.57–2009.89]. Furthermore, these data suggest that this lineage was introduced in the NICU in CH1 in late in 2017 [95% HPD, 2016.94–2017.36] (Figure 6.3C).

Ultimately, the data show that outbreak *Acinetobacter baumannii* ST570 from CH1 were the same lineage and shared a most common ancestor with the organisms isolated from HTD in 2010/2011 (Figure 6.2C), suggesting a previous inter-hospital transmission event. However, it is more likely that ST570 is broadly distributed in ICUs in hospitals in Vietnam and has the ability to longitudinally colonise patients. It seems that this organism has a selective advantage for surviving in such wards and may trigger a blood, or other, infection in those that are vulnerable, young, have comorbidities, or a compromised immune system.

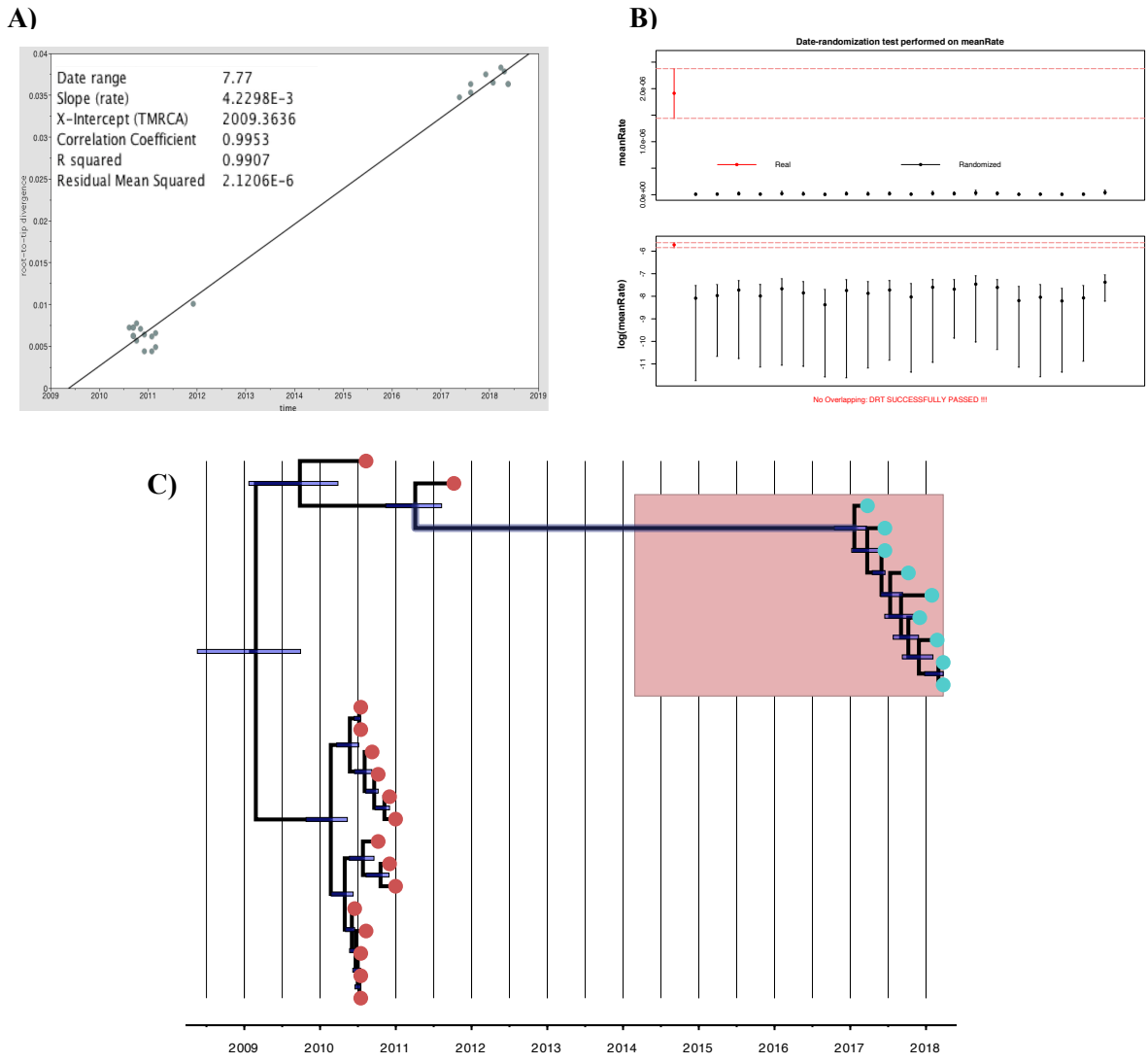

Figure 6.3 Inferring a timescale of a potential *Acinetobacter baumannii* outbreak

A. Linear regression between the date of sampling (x-axis) and the root-to-tip divergence (y-axis) using TempEst to investigate the temporal signal of data. **B.** Validating estimates of mean rates of the correct sampling time and the randomized- dated data sets. **C.** Bayesian phylogenetic reconstruction of ST570 using 121 core SNPs of 25 isolates with the corresponding temporal timescales outputting from BEAST analysis. Tip colours: red (isolates from previous study), light blue (isolates in this study).

6.3 Discussion

Using MLST analysis, I found that 80% of *Acinetobacter baumannii* (12/15 isolates) were sequence type ST570, which belongs within genomic complex 2 (GC2), the remainder 13.3% (2/15 isolates) were ST164. Sequence type was not determined in one isolate as this is a new sequence type that has not been described in the MLST database of *Acinetobacter baumannii*. These results are consistent with a case study investigating the evolution of *Acinetobacter baumannii* in a single ICU with patients having extensive burn wounds and prolonged MDR *Acinetobacter baumannii* infection (234) of which MLST and SNP analysis of 23 *Acinetobacter baumannii* isolates indicated that a majority with 22/23 of the isolates were GC2 (234). In another study using whole-genome sequencing to analyse the emergence of carbapenem-resistant *Acinetobacter baumannii* causing ventilator-associated pneumonia (VAP) in the ICU during 2009–2012, most resistant isolates were global clone GC2 with ST2 and its single locus variants: ST570 and ST571 (150).

All of isolates in this study within lineage ST570 contained $bla_{\text{OXA-23}}$, which encodes resistance to carbapenems. In a study of carbapenem-resistant *Acinetobacter baumannii* causing ventilator-associated pneumonia in the ICU during 2009–2012, most of resistant isolates were global clone GC2 with the presence of gene *oxa23* (150). The *oxa23* gene encoding the OXA-23 carbapenemase is widespread in *Acinetobacter baumannii* isolates and causing treatment failure with carbapenems (233). This gene is known to derive from the chromosome of *Acinetobacter radioresistens* (*oxaAr*) where it is an intrinsic gene(233).

In this study, the *bla*$_{\text{OXA-23}}$ gene was generally located in a gene cassette within an ISAba1 insertion sequence, which belongs to the IS4 transposase family and is commonly found within clinical isolates of *Acinetobacter baumannii*. It has been observed that in *Acinetobacter baumannii*, *oxa23* is located within ISAba1, which supplies the strong promoter leading to high level drug resistance (233).

Carbapenem resistance was associated with the presence of *oxa23* gene in multiple lineages from *Acinetobacter baumannii* causing VAP in a study at an ICU between 2009 and 2012 (150). Phylogenomic analysis in this study revealed five distinct GC2 sublineages within the ICU that had evolved locally via independent chromosomal insertions of *oxa23* transposons (150). The increase in carbapenem-resistant *Acinetobacter baumannii* with VAP was suggested to be associated with transposon-mediated transmission of a carbapenemase gene (150).

Resistance genes that are responsible for resistance to beta-lactam antimicrobials, including *bla*$_{\text{MBL}}$, *bla*$_{\text{ADC}}$, and *bla*$_{\text{A2}}$ were found in most of isolates in this location. These resistance genes were also found in a study drafting the genome sequences of two different carbapenem-resistant *Acinetobacter baumannii* isolates from the blood culture of 10-year-old girl with a burn injury hospitalized at a local hospital in Iran in 2012 (235). Further, I found that genes conferring resistance to macrolides, namely *mphE* and *msrE*, were present in all of the ST570 isolates and 11/12 (91.6%) of these isolates additionally carried the *armA* gene, encoding resistance to aminoglycosides. A comparative analysis of three *Acinetobacter baumannii* genomes revealed that three recently isolated organisms from Beijing acquired these drug-resistance genes into their genomes leading to MDR (236).

Notably, I found that an outbreak *Acinetobacter baumannii* ST570 from CH1 was the same lineage and had a common ancestor with the organisms isolated in an adult ICU in alternative hospital in Vietnam in 2010/2011. This finding could possibly be explained by an inter-hospital transmission event that happened previously. Alternatively, ST570 may be broadly distributed in ICUs in hospitals across Vietnam and is able to longitudinally colonise wards and be easily transferred between hospitals. In a study of *Acinetobacter baumannii* causing VAP in adult ICU during 2009–2012, there were evidence of homologous recombination creating diversity within the local GC2 population, including several events resulting in replacement of the capsule locus. The authors likely found donors of imported capsule locus sequences in the *Acinetobacter baumannii* isolated on the same department; therefore, it is plausible that diversification was caused by the reassortment and sharing of genes between the local *Acinetobacter baumannii* isolates (150).

6.4 Conclusion

In conclusion, I found that most of *Acinetobacter baumannii* isolated in neonatal sepsis at CH1 between January 2017 and June 2018 were sequence type ST570, which belongs within GC2. The genome sequencing revealed the presence of multiple resistance genes which encode resistance to many commonly used antimicrobials, including the key antimicrobials in this location. The $bla_{\text{OXA-23}}$ gene was located in a gene cassette within an ISAba1 insertion sequence, which belongs to the IS4 transposase family and this particular transposon has the potential to be horizontal transfer between organisms leading to widespread carbapenem resistance in *Acinetobacter baumannii*. A potential outbreak of *Acinetobacter baumannii* ST570 from CH1 was the same lineage and shared a most common ancestor with the organisms isolated from HTD between 2010 to 2011, proposing either an inter-hospital transmission previously or a broad distribution in ICUs in hospitals in Vietnam.

7 General discussion

Neonatal sepsis has globally become a key subject in global health. However, scientists and clinicians in SEA are not convinced that our attempts to understand the disease and improve the outcomes are close to where they need to be to fight against the challenges of antimicrobial resistance, early diagnosis, and appropriate treatment. The burden of neonatal infection varies by geographic region, but it is estimated that >1.4 million neonatal deaths occur as the result of infection each year worldwide (4). In LMICs, neonatal sepsis is the most common cause of death in neonates, followed by perinatal asphyxia, congenital anomaly and/or prematurity (192).

Empirical antimicrobials are initiated immediately when neonatal sepsis is suspected or proven. Prompt antimicrobial therapy as recommended by standard policy is important to save lives (54,58). However, resistance to available antimicrobials is currently emerging and constitutes an important problem in SEA, and for this issue, the practice of early and empirical treatment should be based on bacterial profile and local patterns of AMR. The potential risk of untreatable AMR and sepsis in neonates is a major concern in developed countries. However, based on emerging data about AMR in neonatal sepsis from SEA it is now acknowledged that neonatal sepsis caused by MDR Gram-negative bacteria that cannot be effectively treated with available antimicrobial agents has always been an endemic and widespread problem in this region.

Here, I retrospectively described the microbiology of bacteria isolated from the blood culture of children between 2013 and 2016. Consequently, in a prospective study (138), I investigated the clinical features of neonatal sepsis and factors that were associated with

the mortality of patients at CH1 in HCMC in Vietnam from January 2017 to June 2018. I identified the bacterial profile and the antimicrobial susceptibility of bacteria isolated from the blood culture of neonates with sepsis. I characterised the genomics of *Acinetobacter baumannii* causing neonatal sepsis. The results of my DPhil study can be summarised in eight major outcomes:

1) The positivity rate of blood culture at CH1 was 7.6% (1,050/13,727) in 2013, 8.1% (1,086/13,444) in 2014, 7.4% (1,180/15,888) in 2015, and 8.0% (1,379/17,294) in 2016. In my retrospective study, I identified the bacterial profile of BSIs at CH1 from 2013 to 2016 of which the most common bacteria isolated from blood culture in children of all ages were *Acinetobacter* spp., *Escherichia* coli, *Klebsiella* spp., *Pseudomonas* spp., CoNS, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. These organisms showed high AMR to many front-line antimicrobials. My findings were in consistence with results of other studies showing that common bacterial pathogens with high AMR were responsible for BSIs in children (237,238). Practically, CH1 showed high percentages of important MDR bacterial isolates; therefore, infection prevention and control measures and further studies for appropriate empirical antimicrobial therapy are needed.

2) Theoretically based on the available lowest susceptibility rates of bacteria isolated from blood culture 2013–2016, I found that ampicillin, ceftazidime, cefepime, and oxacillin provided limited coverage against major bacteria in the treatment of BSIs at CH1 between 2013 and 2016. This was an alarming issue in the context that imipenem and vancomycin were the last resort for BSIs at CH1. The unbalance between MDR bacteria and new antimicrobial agents has been recognised in other studies (239) and this issue leads to a demand of effective antimicrobial stewardship programmes in children's hospitals (240).

In our practice at CH1, we may solve these problems by optimising the dosing and usage of available antibiotics and the judicious use of common antimicrobials is essential to save the last-resorts drugs for severe cases while waiting for new generations of antimicrobials.

3) In a prospective study at CH1 from January 2017 to June 2018, there were various and non-specific clinical and laboratory presentation in 524 patients with neonatal sepsis of which severe sepsis (23.1%; 121/524) and septic shock (13.9%; 73/524) were observed. The majority of cases were diagnosed and intensively treated as LOS in 91.4% (479/524) and HAS in 73.3% (384/524) of all patients. The mortality was 13.2% (69/524) of all study participants. The clinical picture of neonatal sepsis at CH1 was similar to the findings of other studies of which the signs and symptoms of sepsis in neonates are various and non-specific ranging from few initial manifestations to later severe complications of sepsis including respiratory failure, cardiovascular collapse and multiple organ dysfunction (62). As the clinical features of sepsis are nonspecific, early diagnosis and prompt treatment remains a challenge in neonatal population (241). In CH1, we aim to identify patients with neonatal sepsis early for the perspectives of easier treatment and more likely recovery. It is crucial for paediatricians and neonatologists to remain alert for the clinical indicators of sepsis in neonates and then the appropriate and prompt management in severe cases is required.

4) From data of 524 neonates with 69 deaths (mortality rate of 13.2%) at CH1 from January 2017 to June 2018, factors including sclerema, leukopenia <4,000/mm^3, thrombocytopenia <100,000/mm^3, base excess < –20 mEq/L, serum lactate >4 mmol/L, and hyperglycaemia >180 mg/dL were found to be potentially associated with mortality of patients. Sclerema (196,210), leukopenia (212,213), thrombocytopenia (199,200),

hyperglycaemia (201) and severe acidosis (202) have been found to increase the mortality of patients with sepsis. The associations between these factors and the mortality of neonates with sepsis suggest practical directions for clinicians at CH1 in terms of early recognition, close monitoring, and timely intensive care in order to improve the outcome of patients.

5) I identified 405 organisms including 133 Gram-negative bacteria (32.8%) and 272 Gram-positive bacteria (67.2%) isolated from the blood culture in neonatal sepsis at CH1 from January 2017 to June 2018. The main bacteria were *Klebsiella* spp. (28/405, 6.9%), *Escherichia coli* (27/405, 6.7%), *Acinetobacter* spp. (16/405, 4.0%), and *Enterobacter* spp. (14/405, 3.5%), CoNS (232/405, 57.3%), *Staphylococcus aureus* (18/405, 4.4%), and *Streptococcus* spp. (10/405, 2.5%). These pathogens of neonatal sepsis showed remarkably high non-susceptibility rates to cephalosporins, quinolones, oxacillin and aminoglycosides. Especially, MDR and XDR were found with *Acinetobacter baumannii*. Our results were similar to the findings of other studies in LMICs where the global challenge of AMR and its burden have been emphasized in the neonatal population (242,243). In our practice, routine investigation of bacterial profile and antimicrobial susceptibility patterns has been a fundamental component in the management of neonatal sepsis. These databases are essential for the development of appropriate antimicrobial usage guidelines at CH1.

6) The antimicrobial susceptibility patterns of isolated bacteria in neonatal sepsis at CH1 from January 2017 to June 2018 lead to an observation that our empirical therapy with non-carbapenem antimicrobials provided limited coverage for many bacterial pathogens of neonatal sepsis. This result was similar to the finding of a study that non-carbapenems

may provide limited empirical coverage in neonatal sepsis in Asia (244). It was probable that sepsis in neonates during the study period could only be treated effectively with carbapenems (imipenem, meropenem) and vancomycin. For the clinical application at CH1, the recommendation of ampicillin and cephalosporins may no longer be valid for several cases of severe sepsis in neonates. Therefore, the assessment of antimicrobial alternatives will be crucial to reduce the use of last-resort drugs for the empirical treatment of neonatal sepsis in CH1.

7) During the course of the prospective study at CH1 from January 2017 to June 2018, I isolated 15 *Acinetobacter baumannii* from the blood of neonates, I found that the most of isolates (12/15; 80%) were sequence type ST570, which belongs within genomic complex 2 (GC2), and the remainder (2/15; 13.3%) were ST164. Beta-lactam antimicrobials resistance genes including bla_{MBL}, bla_{ADC}, and bla_{A2} were found in most of isolates. Additionally, all of isolates within lineage ST570 contained $bla_{\text{OXA-23}}$ encoding resistance to carbapenems. The particular transposon carrying the $bla_{\text{OXA-23}}$ has been identified a common transposon and has the potential to be horizontal transfer between organisms, consequently leading to widespread carbapenem resistance in *A. baumannii* found in hospital infection. I also found macrolides resistance genes including *mphE* and *msrE* in all ST570 isolates and 11/12 (91.6%) of these isolates additionally carried the *armA* gene, encoding resistance to aminoglycosides. Similar to the findings of a study on *Acinetobacter baumannii* in a single ICU in Vietnam, our results emphasise the importance of genomics analysis to study the AMR genes of this important pathogen (150).

8) Ultimately, *Acinetobacter baumannii* ST570 from CH1 were the same lineage and shared a most common ancestor with the organisms isolated in 2010/2011, suggesting an inter-hospital transmission event previously. More likely, is that ST570 is broadly distributed in ICUs in hospitals in Vietnam and has the ability to longitudinally colonise wards. It seems that this organism has a selective advantage for surviving in such wards and may trigger a blood, or other, infection in those that are vulnerable, young, have comorbidities, or a compromised immune system. Practically, any treatments of sepsis with *Acinetobacter baumannii* ST570 (e.g., monoclonal antibody therapy) can potentially be applied to all patients in several ICUs in hospitals in Vietnam on the basis of the observation that these organisms were the same lineage and shared a most common ancestor.

The strength of my study is that it contributes to the knowledge of scientists and clinicians in the setting that we know very little is known about the current aetiological agents and their antimicrobial susceptibility patterns of neonatal sepsis in Vietnam. Consequently, the clinical, microbiological, and genomic data produced by my study will be essential information for clinical practice and the development of future guidelines. This study was conducted at the largest tertiary paediatric hospital in southern Vietnam. In this study, I integrated the clinical assessments with the microbiology and detailed genomics data to further characterize the features of neonatal sepsis and in this high mortality setting.

My study has several limitations. Firstly, I found ethical issues complicated the way we conduct research in severely ill neonates. Secondly, there were no systematic guidelines ensuring accurate diagnosis and management of sepsis at CH1. Thirdly, the number of cases showed stochastic variations. Fourthly, continuous changes and lack of consensus

in antimicrobial therapy were limitations. Fifthly, it was not practical for us to investigate the clinical features of children with BSIs in the retrospective study. Finally, data collection at a single paediatric hospital limits the applicability of my results to other places in Vietnam.

My study has found that sepsis is an obvious cause of morbidity and mortality of neonates in SEA. Generally, the three main issues of neonatal sepsis that turn out to be indispensable are the requirement of state-of-the-art laboratory tools for better and early diagnosis, the antimicrobial resistance crisis, and the strive for effective nosocomial infection prevention and control. Future research should focus on validity and applicability of proteomics-based experiments, molecular techniques or better markers of inflammation in order to develop rapid diagnostic tests for early detection of neonatal sepsis, its pathogenic bacteria, and the AMR patterns. With highly sensitive and specific diagnostic tools, the treatment of neonatal sepsis would be significantly changed and so that antimicrobial therapy could be safely and comprehensively administered or withheld. All of these may lead to costs reduction and the improvement of overall outcomes of neonatal sepsis.

With our clinical and laboratory capacity at CH1, specific studies in near future might aim to focus on research outcomes including a comprehensive understanding of the microbiological and genetic makeup and the temporal and phylogenetic relationships of bacteria causing BSIs at CH1, a baseline of data permitting prospective studies aimed at detecting bacteraemia cases more rapidly (i.e. by PCR on blood), a baseline of data permitting prospective studies to detect patients early with MDR infections of those with infections with bacteria containing AMR genes, a data resource that will be made

available to clinicians working at CH1 managing BSIs, and a measure of evolutionary rate, antimicrobial resistance development and the role of the accessory genome in the local evolution of the causes of bacteraemia at CH1.

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9 Appendices

9.1 The approval of The Oxford Tropical Research Ethics Committee (OxTREC)

The ethics reference number of my study is OxTREC 35-16 (Oxford, England).

Oxford Tropical Research Ethics Committee

University of Oxford
Research Services, University Offices
Wellington Square, Oxford OX1 2JD
Tel. +44 (0) 1865 (2)82106
E-mail: oxtrec@admin.ox.ac.uk

UNIVERSITY OF OXFORD

Professor Stephen Baker
Oxford University Clinical Research Unit
The Hospital for Tropical Diseases
Ho Chi Minh City, Vietnam

04 August 2016

Dear Professor Baker

Full Title of Study: **The clinical features, antimicrobial susceptibility patterns and genomics of bacteria causing neonatal sepsis in a children's hospital in Vietnam**

OxTREC Reference: 35-16

Thank you for your letter of 3rd August 2016 in which you have responded to the Committee's request for further clarification.

I am pleased as Chairman for OxTREC to give approval for this study. This is valid for the first five years and is subject to receiving the local ethical approval (if this approval has not yet been received).

The documents approved for this study are as follows:

Documents:	Version:	Date:
Protocol	2.0	03AUG16
ICF	1.4	03AUG16

Any subsequent changes to the application must be submitted to the Committee as an Amendment. This should include a letter to give the reasons for the proposed modifications and all revised documents with changes tracked.

Please ensure that you submit a completed Annual Report form on every anniversary of this approval and a final End of Study Report. The relevant forms can be found on the OxTREC website: http://www.tropicalmedicine.ox.ac.uk/oxtrec.

Yours sincerely

Dr Mary Warrell
OxTREC Chairman

Direct Line Tel: +44 (0)1865 (2)82106
Email: oxtrec@admin.ox.ac.uk
Web: www.admin.ox.ac.uk/rso/

Oxford Tropical Research Ethics Committee

University of Oxford
Research Services, University Offices
Wellington Square, Oxford OX1 2JD
Tel. +44 (0)1865 (2)82106
E-mail: oxtrec@admin.ox.ac.uk

09 August 2017

Professor Stephen Baker
Oxford University Clinical Research Unit
Centre for Tropical Medicine
764 Võ Văn Kiệt, Quận 5
Ho Chi Minh City, Viet Nam

Dear Professor Baker

Full Title of Study: The clinical features, antimicrobial susceptibility patterns and genomics of bacteria causing neonatal sepsis in a children's hospital in Vietnam (19EN)

OxTREC Reference: 35-16

Thank you for your letter of the 4 August 2017 requesting an amendment to the above study. The revised documents that you have submitted for consideration are as follows:

Documentation:	Version:	Date:
Protocol	2.1	08JUN17
ICF	1.5	04AUG17

I am pleased to inform you that this amendment has been approved.

Yours sincerely

Dr Rebecca Bryant
Research Ethics Manager, OxTREC

Direct Line Tel: +44 (0)1865 (2)82106
Email: oxtrec@admin.ox.ac.uk Web: www.admin.ox.ac.uk/rso/

Oxford Tropical Research Ethics Committee

University of Oxford
Research Services, University Offices
Wellington Square, Oxford OX1 2JD
Tel. +44 (0) 1865 282585
E-mail: oxtrec@admin.ox.ac.uk

UNIVERSITY OF OXFORD

To whom it may concern 10 August 2016

Dear Sir/Madam

Title: The clinical features, antimicrobial susceptibility patterns and genomics of bacteria causing neonatal sepsis in a children's hospital in Vietnam.

OxTREC Ref: 35-16

The above study has been designed by Professor Stephen Baker and colleagues at the University of Oxford. It is funded by the Royal Society and the Wellcome Trust. I confirm that the University will accept responsibility for the overall initiation, management and/or financing of the study, subject to all local ethical and regulatory requirements being met.

The University maintains appropriate insurance in the territories in which the study will be conducted; to the extent that a claim is made against the University, this will apply to this study.

Sponsorship is confirmed subject to the condition that should any substantial amendments or reports be submitted to the Local Ethics Committee, these should also be copied to OxTREC.

Yours faithfully

Dr Richard Liwicki
Deputy Director, Research Services

9.2 The approval of The Ethics Committee of Children's Hospital 1 in Vietnam

The ethics reference numbers of my study are 73 and 122/GCN-BVND1 (Vietnam).

SỞ Y TẾ
THÀNH PHỐ HỒ CHÍ MINH
BỆNH VIỆN NHI ĐỒNG 1
Số: 73 / GCN-BVNĐ1

CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập – Tự do – Hạnh phúc

Thành phố Hồ Chí Minh, ngày 08 tháng 7 năm 2016

GIẤY CHỨNG NHẬN
Chấp thuận của hội đồng đạo đức trong nghiên cứu y sinh học
Bệnh viện Nhi Đồng 1

Căn cứ quyết định số 117/QĐ – BVNĐ1 của Giám đốc Bệnh viện Nhi Đồng 1 về việc thành lập Hội đồng khoa học và đạo đức trong nghiên cứu y sinh học nhiệm kỳ 2012 – 2016;

Căn cứ quyết định số 1123/QĐ – BVNĐ1 ngày 22/6/2016 của Giám đốc bệnh viện về việc thành lập Hội đồng xét duyệt đề cương nghiên cứu khoa học Đợt 3 năm 2016;

Căn cứ biên bản họp số 450/BB-BVNĐ1 ngày 8/7/2016 của HĐĐĐ đánh giá các khía cạnh đạo đức của đề tài nghiên cứu khoa học.

Hội Đồng Khoa Học và Đạo Đức trong Nghiên cứu Y sinh học Bệnh viện Nhi Đồng 1 (IORG0007285, FWA00009748), Ban Giám Đốc bệnh viện Nhi Đồng 1

Chấp thuận cho thực hiện đề tài:

"Đặc điểm lâm sàng, sự nhạy cảm kháng sinh và di truyền học vi khuẩn trong nhiễm khuẩn huyết sơ sinh"

Với các tài liệu sau:

- Protocol V1.9 ngày 29/5/2016
- ICF V1.3 ngày 29/5/2016

Mã số nghiên cứu: CS/N1/16/26 (19EN)

Chủ nhiệm đề tài: BS Nguyễn Đức Toàn

Thời gian nghiên cứu: tháng 8/2016 đến tháng 12/2020

Phương thức xét duyệt: theo quy trình đầy đủ

Đề nghị Chủ nhiệm đề tài, nhóm nghiên cứu và Nhà tài trợ phải tuân thủ đúng theo Hướng dẫn Thực Hành tốt lâm sàng (GCP Guidelines) và nội dung đề cương nghiên cứu đã được phê duyệt, đảm bảo tuyệt đối an toàn cho bệnh nhân tham gia nghiên cứu.

Ngoài ra cần lưu ý một số điểm sau:

- Bất kỳ những thay đổi, sai lệch hay chỉnh sửa đề cương đã được phê duyệt và mẫu bản chấp thuận tham gia nghiên cứu cần phải có xem xét và chấp thuận của HĐĐĐ;

- Báo cáo tiến độ ít nhất mỗi năm một lần và theo yêu cầu của HĐĐĐ;

- Thông báo về việc ngừng nghiên cứu, kết thúc nghiên cứu trước thời hạn dự kiến, lý do của việc kết thúc sớm;

- Chuẩn bị cho việc kiểm tra điểm nghiên cứu của HĐĐĐ.

Hội đồng đạo đức trong nghiên cứu y sinh học Bệnh viện Nhi Đồng 1 đã dựa trên nguyên tắc thực hành lâm sàng tốt ICH-GCP cũng như những quy định hiện hành trong quá trình xem xét và phê duyệt các đề tài thử nghiệm lâm sàng tại Bệnh viện.

Trân trọng kính chào./.

Nơi nhận:
-Chủ nhiệm đề tài;
-Lưu:VT, NCKH-MD.

GIÁM ĐỐC

SỞ Y TẾ THÀNH PHỐ HỒ CHÍ MINH
BỆNH VIỆN NHI ĐỒNG 1

Nguyễn Thanh Hùng

SỞ Y TẾ
THÀNH PHỐ HỒ CHÍ MINH
BỆNH VIỆN NHI ĐỒNG 1

Số: 122 / GCN-BVNĐ1

CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập – Tự do – Hạnh phúc

Thành phố Hồ Chí Minh, ngày 31 tháng7 năm 2017

GIẤY CHỨNG NHẬN

Chấp thuận của Hội đồng đạo đức trong nghiên cứu y sinh học Bệnh viện Nhi Đồng 1 đối với các bổ sung trong nghiên cứu

---

Căn cứ quyết định số 117/QĐ – BVNĐ1 của Giám đốc Bệnh viện Nhi Đồng 1 về việc thành lập Hội đồng khoa học và đạo đức trong nghiên cứu y sinh học nhiệm kỳ 2012 – 2016;

Căn cứ quyết định số 1187/QĐ – BVNĐ1 ngày 10/7/2017 của Giám đốc bệnh viện về việc thành lập Hội đồng xét duyệt đề cương nghiên cứu khoa học Đợt 6 năm 2017;

Căn cứ đơn đề nghị ngày 12/6/2017 của đại diện nhóm nghiên cứu – ThS. BS Nguyễn Đức Toàn về việc thẩm định, xét duyệt cập nhật Đề cương phiên bản 2.1 và Phiếu chấp thuận tham gia nghiên cứu V1.5,

Hội Đồng Khoa Học và Đạo Đức trong Nghiên cứu Y sinh học Bệnh viện Nhi Đồng 1 (IORG0007285, FWA00009748),

Chứng nhận thông qua các thay đổi, bổ sung đối với đề tài:

"Đặc điểm lâm sàng, sự nhạy cảm kháng sinh và di truyền học vi khuẩn trong nhiễm khuẩn huyết sơ sinh".

Mã số nghiên cứu: CS/N1/16/26

Chủ nhiệm đề tài: ThS.BS Nguyễn Đức Toàn

Nội dung thay đổi, bổ sung được thông qua như sau:

STT	Tên tài liệu	Phiên bản/Ngày
1	Đề cương nghiên cứu	**2.1** Ngày 08/6/2017
2	Phiếu thông tin và chấp thuận tham gia nghiên cứu	**1.5** Ngày 08/6/2017

Chủ nhiệm đề tài, nhóm nghiên cứu và Nhà tài trợ phải tuân thủ đúng theo Hướng dẫn Thực Hành tốt lâm sàng (GCP Guidelines) và nội dung đề cương nghiên cứu đã được phê duyệt, đảm bảo tuyệt đối an toàn cho bệnh nhân tham gia nghiên cứu.

Trong quá trình thực hiện nghiên cứu, nhóm nghiên cứu phải báo cáo tiến độ hàng quý cho Hội đồng. Sau đó, Hội đồng sẽ tiến hành giám sát, kiểm tra định kỳ 3 tháng/lần.

(Đính kèm Bảng tóm tắt những thay đổi trong đề cương và phiếu chấp thuận)

Trân trọng kính chào./.

Nơi nhận:
-Chủ nhiệm đề tài, ĐV NCLS ĐH Oxford;
-Lưu:VT, CĐT-MD (03).

CHỦ TỊCH HỘI ĐỒNG

SỞ Y TẾ THÀNH PHỐ HỒ CHÍ MINH
BỆNH VIỆN NHI ĐỒNG 1

Phó Giám đốc
Lê Bích Liên

9.3 The registration with the International Standard Randomized Controlled Trial Number (ISRCTN) registry

My study is listed on the ISRCTN registry with study ID ISRCTN69124914.

From: info@isrctn.com
Subject: Study The clinical features, antimicrobial susceptibility patterns and genomics of bacteria causing neonatal sepsis in a children's hospital in Vietnam 33632 registered in ISRCTN Register with ISRCTN69124914
Date: June 6, 2017 at 4:40:05 PM GMT+7
To: toanped@gmail.com

Dear trialist,

We are pleased to inform you that the study entitled "The clinical features, antimicrobial susceptibility patterns and genomics of bacteria causing neonatal sepsis in a children's hospital in Vietnam" is now listed on the ISRCTN registry with study ID ISRCTN69124914.

You can view your study at: http://www.isrctn.com/ISRCTN69124914

Thank you for registering your study with the ISRCTN registry.

When quoting the ISRCTN, please make sure that no space is inserted between the ISRCTN and the actual number. Please refer to http://www.isrctn.com/page/faqs#usingISRCTN for further guidance about how to use the ISRCTN.

Please also note that once a study has been registered on the ISRCTN registry and publicly displayed on the website, the study will remain permanently on the registry and cannot be deleted.

If you wish to update your ISRCTN record, please send an email to info@isrctn.com. Please note that we cannot remove information from a record, or overwrite previous information, but will instead add any updated information, along with a date stamp to show when the changes were made to the study record. Please see http://www.isrctn.com/page/faqs#checkingRecord for further information.

The ISRCTN registry is administered and published by BioMed

Central which also currently publishes over 280 peer-reviewed open access journals. These journals include Trials and Pilot and Feasibility Studies, as well as the BMC series medical journals, which offer a platform for publishing both protocols and research articles relating to clinical trials.

UK only - The UK National Institute of Health Research (NIHR) has signed up for a Supporter Membership which helps NIHR-funded researchers to publish their work in any of the BioMed Central journals (http://www.biomedcentral.com/publishing-services/membership/members/1600002969).

Kind regards,

ISRCTN Editorial Team

c/o BioMed Central

Floor 6, 236 Gray's Inn Road

London WC1X 8HB

UK

info@isrctn.com

Follow @ISRCTN on Twitter

9.4 The protocol for the observational study

Protocol No

19EN

Project

The clinical features, antimicrobial susceptibility patterns and genomics of bacteria causing neonatal sepsis in a children's hospital in Vietnam

Principal Investigators

Stephen Baker (Oxford University Clinical Research Unit)

Nguyen Duc Toan (Children's Hospital 1)

Investigators

Thomas Darton (Oxford University Clinical Research Unit)

Nguyen Thanh Hung (Children's Hospital 1)

Ngo Ngoc Quang Minh (Children's Hospital 1)

Le Nguyen Thanh Nhan (Children's Hospital 1)

Nguyen Kien Mau (Children's Hospital 1)

Pham Thi Thanh Tam (Children's Hospital 1)

Le Quoc Thinh (Children's Hospital 1)

Collaborating Institutions

Oxford University Clinical Research Unit (OUCRU), Ho Chi Minh City, Vietnam

Children's Hospital 1 (CH1), Ho Chi Minh City, Vietnam

Introduction

Background

Global concerns regarding antimicrobial resistance (AMR) in bacterial infections have recently been reinvigorated (245). This resurgence in a once neglected area has been fuelled mainly by the renewed focus on multi-drug resistant (MDR – resistance to several groups of antimicrobials) hospital acquired infections with pathogens such as *Staphylococcus aureus* (246) and *Acinetobacter baumannii* (247) in high-income countries. The potential risk of untreatable bacterial infections is a major concern in industrialised countries. Based on emerging AMR data from across the developing world it is now acknowledged by the World Health Organization that drug resistance has also become an endemic and widespread problem in low-income countries (129). In many low-income countries, untreatable bacterial infections with broadly AMR pathogens are no longer a threat but an unwelcome reality. Therefore, AMR in low-income countries represents one of the biggest threats to global health and is one of the greatest current challenges in infectious disease research.

The trend of increasing AMR in low-income countries is particularly worrying because of the significantly greater impact of these pathogens in resource-limited areas of the world. The reasons behind the apparent amplification of the AMR threat in lower income countries are complex, but there are common themes: 1) Bacterial pathogens found in lower income settings typically cause more severe infections than those in higher-income countries (e.g. typhoid fever), 2) Antimicrobials are widely available for purchase in the community without medical consultation, 3) Medical treatment and healthcare facilities are generally not as good in lower income countries as in rich countries, and 4) Very few patients receive any form of conclusive diagnostic testing

(e.g. such as bacterial isolation and antimicrobial susceptibility testing) before, or indeed after, they are treated with an empiric antimicrobial regimen (typically fluoroquinolones or cephalosporins for febrile infections). It is these factors, which appear to drive AMR and make novel AMR strains of bacteria more likely to originate in lower-income countries. This is well illustrated by the first reports of the carbapenem resistance gene New Delhi Metallo-beta-lactamase-1 (NDM-1), which was first reported from a *Klebsiella pneumoniae* isolate in India in 2008 (248).

AMR is an issue with all types of bacterial infections, yet BSIs are the most dangerous and the rapid detection and treatment of an AMR pathogen in the blood may mean the difference between life and death. BSIs can demonstrate extensive geographical diversity in both aetiology and the proportions of isolated bacteria that exhibit resistance to antimicrobials (130,249). An understanding of the epidemiology of BSIs in hospitalized neonates is crucial in the development of rational management and treatment guidelines.

Bacterial BSIs can be generally classified into two major groups, community acquired and hospital acquired, according to their place of acquisition. AMR is an issue for both types of infection, although it is currently among hospital acquired infections that the most resistant isolates are appearing.

Hospital-acquired bacterial BSIs are a major threat to patient safety, and in locations with poor surveillance and infection control programmes such infections can be associated with high mortality rates. The problem of nosocomial transmission of hazardous bacteria in low-income countries is exacerbated by this current AMR surge, particularly to 3rd/4th generation cephalosporins and carbapenems in Gram-negative bacteria and to

vancomycin and methicillin in Gram-positive bacteria. Our recent work in Nepal exemplifies this issue as we observed two outbreaks of MDR *Klebsiella pneumoniae* encompassing 48 children with a mortality rate of 75% (136). Additionally, we have found that the incidence of AMR BSIs across three hospitals in Ho Chi Minh City, Vietnam has dramatically increased over recent years, and is predicted to increase further. This trend appears to be fuelled by increased isolation rates of both Gram-negative and Gram-positive bacterial infections (chiefly *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus* and Enterococci) that are resistant to a wide spectrum of antimicrobials, including many "last resort" drugs.

Community acquired bacterial BSIs are also an important cause of fever among patients admitted to hospital across South and Southeast Asia. Distinguishing bacterial infections from other causes of fever, such as malaria or viral infections including dengue, can be challenging without diagnostic laboratory support (250). Important causes of community-acquired infections include *Salmonella* serovars Typhi and Paratyphi A, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. We have reported increasing levels of AMR in each of these common pathogens and highlighted the difficulties of accurate diagnosis with traditionally available diagnostics. For example in Nepal and Vietnam drug resistance in *Salmonella* Typhi and *Salmonella* Paratyphi A has severely restricted the options available for antimicrobial treatment (133).

A study published in 2012 highlighted the changing aetiology of BSIs at HTD over fifteen years. In this study, *Salmonella* Typhi was the dominant bloodstream pathogen from 1994 to 2002, after which it declined dramatically (134). Since 2002, non-typhoidal *Salmonella* and other opportunistic HIV-associated pathogens including *Cryptococcus neoformans*

and *Penicillium marneffei* have replaced *Salmonella* Typhi as the leading agents of BSIs. This shift is likely to reflect a changing landscape of infectious disease related to the HIV epidemic, urbanization and secondary social determinants within Vietnam. Vietnam, as with many countries in Asia, is undergoing a rapid economic transition, and programmes to improve sanitary conditions have reduced the overall risk of water-borne infections. HIV-associated opportunistic pathogens have now emerged as the leading pathogens of BSIs and the primary cause of mortality in hospitalized adult patients in this location. These studies focussed mainly on adults, including those with HIV, and children but not neonates. The observations may not be fully representative of the situation in children, as many are seen at the Children's Hospitals rather than HTD, and neonates, which are not admitted to HTD but where the impact of AMR can be devastating.

We know little about contemporary antimicrobial susceptibility patterns (and their corresponding antimicrobial resistance genes) in the major bacterial causes of BSIs in Vietnam and how these impact on disease and outcome. To address this issue, we wish to study the distribution of antimicrobial resistant pathogens at CH1 causing BSIs in neonates. This includes studying resistance to third generation cephalosporins, fluoroquinolones, and carbapenems in Gram-negative organisms and methicillin and vancomycin in *Staphylococcus*. This will be achieved by prospectively studying the bacteria causing bacteraemic infections in neonates at CH1. The antimicrobial susceptibility of those isolates by disc testing and minimum inhibitory concentrations. The archived isolates will be later examined by conventional PCR and genome sequencing of resistance genes.

PCR amplification will be used to define the presence/absence of the major gene groups catalysing antimicrobial resistance (i.e., detect previously described genes). Genome sequencing will be used to study the phylogenetic relationships between specific organisms and identify the pool of antimicrobial resistance genes circulating in the bacteria causing BSIs (i.e., total pool of antimicrobial resistance genes and to identify new resistance genes). Genome sequencing is now the gold standard for understanding bacterial populations in the greatest definition and essential for publication in high quality international journals.

This will be a clinical and microbiologic research project and collaboration between CH1 and OUCRU-VN. This study will be a conduit for introducing molecular biology for bacteriology into routine hospital care at CH1 and will lead to future studies investigating appropriate empirical treatment for bacteraemia and the impact of antimicrobial resistance on the outcome of BSIs in the paediatric population in Vietnam.

Scientific rationale

There are limited contemporary data on the causes of bacterial sepsis in neonates in Vietnam. We hypothesize that there has been a dramatic surge in multi-drug resistant Gram-negative organisms and the emergence of MRSA. To understand these important causes of morbidity and to best inform antimicrobial treatment regimens we aim to describe the bacterial aetiology of bloodstream infection in neonates and investigate the prevalence of antimicrobial resistance at CH1 from 2016 to 2019.

We will establish a prospective bloodstream infection surveillance in neonates at CH1 from 2016 to 2019, whereby infecting pathogens are identified, confirmed, antimicrobial susceptibility tested, archived and examined using molecular techniques.

Objectives and outcomes

This is a prospective hospital-based study aiming to determine the bacteriological causes of neonatal sepsis and the associated antimicrobial susceptibility profiles at CH1. Our main interest lies in studying multi-drug resistant (MDR) Gram-negative organisms and methicillin resistant *Staphylococcus aureus* (MRSA) (251).

This study will be performed with a set of routine data available at CH1 from 2016 to 2019. We aim to investigate the clinical features, major causes of neonatal sepsis at CH1, distribution of pathogens by departments, their antimicrobial susceptibility patterns and the genomics of bacteria as well as their association with disease outcome.

We hope that by studying and defining aetiology, antimicrobial susceptibility patterns and disease outcome we are be able to develop an improved approach to managing BSIs and we will use these data to initiate intervention studies focused on preventing BSIs with antimicrobial resistant pathogens in patients in this setting.

We additionally aim to detect metabolites signatures for specific organisms and AMR profiles from patients with BSIs. Metabolomics is a comparatively new methodology in infectious disease research, yet some initial investigations have shown that metabolite signals found in biological samples may have potential as “biomarkers” of infection as well as of AMR. Metabolomics exploits mass spectrometry, or NMR spectroscopy, to

interrogate biological material for potential biomarkers or biomarker patterns of infection. Together with powerful bioinformatics, often based on pattern recognition or chemometric approaches, metabolomics is a highly sensitive tool and can potentially distinguish between different pathogens in the blood. Previous work highlights the sensitivity of the method given that these are closely related organisms and induce a disease phenotype via a relatively modest concentration of organisms in the blood. Scientists at Umeå University Sweden are field leaders in this area.

This project builds upon previously published retrospective studies of BSIs at the Hospital for Tropical Diseases (HTD) in HCMC (134,252–254), which were focused on adults and children but not neonates.

Study objectives

Primary objectives

1. To investigate the clinical characteristics of neonates with sepsis.
2. To define the aetiology, the percentage of positive blood culture and major causes of sepsis.
3. To investigate the antimicrobial susceptibilities of the pathogens causing sepsis and the prevalence of antimicrobial resistance.
4. To measure the impact of sepsis on the severity of disease and the outcomes (mortality rate, length of stay) of hospitalized neonates.
5. To investigate the genome sequences of bacterial strains causing neonatal sepsis.

Secondary objectives

1. To analyse the antimicrobial resistance profiles and gene distribution to add insight into the circulation of bacteria causing nosocomial infections.

2. To study the genes catalysing resistance to the antimicrobials commonly used to treat neonatal sepsis (specifically third/fourth generation cephalosporins, fluoroquinolones and carbapenems).

3. To identify metabolomic profiles of AMR BSIs.

4. To understand the profile of bacteraemia-associated pathogens isolated in children including neonates at CH1, specifically related to the emergence of AMR strains.

Study outcomes

Primary outcomes

1. To describe the clinical characteristics of neonates with sepsis seen at CH1 between 2016 and 2019, including community and hospital-acquired sepsis, early and late-onset sepsis

2. To determine the aetiology of neonatal sepsis and the distribution of pathogens by clinical departments at CH1 between 2016 and 2019.

3. To determine the antimicrobial susceptibility profiles of the bacteria causing neonatal sepsis: and the changing antimicrobial resistance (AMR) patterns with community and hospital-acquired sepsis at CH1 between 2016 and 2019.

4. To analyse the impact of specific bacteria and AMR profile on patient outcomes (mortality, length of stay) of neonates with sepsis at CH1 between 2016 and 2019.

Secondary outcomes

1. To analyse the antimicrobial resistance profiles and gene distribution of isolated bacteria at CH1 to add insight into the circulation of bacteria causing nosocomial infections.

2. To determine the genome sequences of bacterial strains isolated from the blood of neonates with sepsis between 2016 and 2019, to study the population structure, the antimicrobial resistance gene pool, and other horizontally transferred DNA between the bacteria.

3. To compile a unique dataset of metabolite profiles stratified by bacterial species and AMR gene content.

4. To understand and describe the temporal trend in isolation rates of bacteraemia-associated pathogens isolated at CH1 particularly in relation to antimicrobial susceptibility profiles.

Future outcomes after analyses

1. A comprehensive understanding of the microbiological and genetic makeup and the temporal and phylogenetic relationships of bacteria causing neonatal BSIs at CH1.

2. A baseline of data permitting prospective studies aimed at to detecting bacteraemia cases more rapidly (i.e. by PCR on blood).

3. A baseline of data permitting prospective studies to early detect multi-drug resistance bacteria containing AMR genes such as ESBL/AMPC.

4. A data resource for managing sepsis that will be made available to clinicians working at CH1.

5. A measure of evolutionary rate, antimicrobial resistance development and the role of the accessory genome in the local evolution of the bacteria causing bacteraemia at CH1

6. A data resource that can be used to generate rapid diagnostic markers for AMR BSIs.

7. A comprehensive description of the time trend patterns of emerging AMR BSIs occurring at CH1.

Research plan

Study design

This protocol describes a prospective, non-interventional, observational study to characterize the clinical features of neonates with sepsis at the Children's Hospital 1 (CH1) in Ho Chi Minh City (HCMC), Vietnam between 2016 and 2019 and the microbial population structure, antimicrobial susceptibility patterns and the antimicrobial resistance genes of the bacteria causing that sepsis.

All organisms isolated from blood will be stored and archived for molecular characterization. All plasma sampled will be stored and shipped to Umeå University in Sweden for metabolite profiling.

Study sites

1. Children's Hospital 1
2. Oxford University Clinical Research Unit

Study population

Systematic criteria concerning which patients should have blood cultures performed are not defined at CH1. However, in general, we consider that a blood culture is initiated in neonates whose diagnosis is probable sepsis. We use the criteria suggested by the European Medicines Agency (EMA) in 2010 for the diagnosis of probable sepsis and culture-confirmed sepsis in neonates (90). Neonatal sepsis is defined by the EMA experts

as the presence of at least two clinical criteria and at least two laboratory criteria in presence of or as a result of suspected or proven infection (frequently by identifying bacterial bloodstream infection) in neonates (90). All neonates that have the diagnosis of probable sepsis or culture-confirmed sepsis and have blood culture will be included in this project.

We have no reason to believe there will be systematic change in the application of these criteria during the selected time course of the proposed study. Therefore, all patients with or without HIV admitted to the hospital who had a blood culture performed for suspected sepsis will be included in this prospective analysis.

Sample size

In this descriptive study, the sample size is dependent on the number of patients with available data according to the inclusion criteria admitted to CH1 from 2016 to 2019.

Study procedures

Routinely, the investigator records to the paper case report form the data including demographic, clinical and laboratory information of the patients, the date of blood draw, the number of blood culture bottles inoculated, the result of the culture (whether positive or negative) and the susceptibility of the isolate to commonly used antimicrobial agents.

Data from these records are subsequently entered into CliRes Data Management System. These will be source data for this study. The number of patients admitted to the hospital annually will be obtained from hospital records.

Description of participants

Inclusion criteria

Neonates (≤1 month of age) that have the diagnosis of "probable sepsis" or "confirmed sepsis" and have blood culture taken who are an in-patient at CH1 between 2016 and 2019.

Neonates (≤1 month of age) that have the diagnosis of "probable sepsis" or "confirmed sepsis" and have blood culture taken who are an in-patient at our local hospital between 2016 and 2019 will be included in this research.

Confirm the eligibility of patients for the study by asking the following questions:
Is the child one month of age or less? (only continue if yes)
Has the child had a diagnosis of probable sepsis or confirmed sepsis? (only continue if yes)
Has the child had a blood culture? (only continue if yes)
If the child meets all of the above criteria, the child will be recruited into the study.

The patients must have the diagnosis of "probable sepsis" or "confirmed sepsis" (confirmed by blood culture) to be included in this study and these patients are our target. The diagnosis of other conditions which may be the sources of sepsis or associated infections (urinary tract infection, enterocolitis, pneumonia, meningitis etc.) will also be recorded.

Informed consent given by parent or guardian.

Exclusion criteria

No informed consent

Eligibility of participants

a) All doctors and nurses will be informed and trained for this study.

b) People who receive the blood culture at microbiology department and people who work at the neonatal intensive care unit will be informed of the study.

c) These people will be trained to identify eligible patients and how to notify investigators when they identify an eligible participant. The study team can then recruit and enrol the patient.

d) Assess eligibility of the patients.

Confirm the eligibility of patients for the study by asking the following questions:

1. Is the child one month of age or less? (only continue if yes)

2. Has the child had a diagnosis of probable sepsis or confirmed sepsis? (only continue if yes)

3. Has the child had a blood culture? (only continue if yes)

If the child does not meet all of the above criteria, the child does not meet the selection criteria for the study.

Informed consent

Informed consent will be taken by the study staff, all of whom will receive specific training in the study and Good Clinical Practice and will be authorized to take consent by the study principal investigators. The study staff will discuss the study with the accompanying parent/guardian. If both parents are dead or not actively involved in caring

for the child, the main long-term care for the child will be accepted as a guardian and considered able to give consent for the study. Study staff will describe the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants, and alternatives to enrolment. The parent/guardian will be invited to ask questions which will be answered by study staff, and they will be provided with appropriate numbers to contact if they have any questions subsequently.

If the parent/guardian agrees for the child to participate, they will be asked to sign and date an informed consent form. A copy of the patient information sheet and the informed consent form will be given to them to keep. In addition to the procedures above, illiterate signatories will have the Informed Consent Form read to them in the presence of a witness who will sign to confirm this. All patient information sheets and consent forms will be written in the local language and will use terms that are easily understandable.

Baseline demographic and clinical assessment

Data on neonatal BSIs at CH1 from 2016–2019 will be collected to the CRF. These data will include:

a. Dates of birth, admission, discharge, transfer or death

b. Demographic data (gestational age, birth weight, gender, place of birth, place of referral, clinical departments)

c. Clinical characteristics

d. Laboratory and imaging results

e. Diagnosis (age at diagnosis, probable sepsis, confirmed sepsis, community or hospital-acquired sepsis, early or late-onset sepsis, severity, septic shock, associated diseases and infections, etc.)

f. Treatments (antibiotics, respiratory support, surgical intervention, long-term parenteral nutrition, central vascular catheters, etc.)

g. Outcomes (mortality, length of stay, cost of treatment, complications).

Microbiological assessments

Available routine microbiology data on neonatal BSIs at CH1 from 2016–2019. These data will include

a. Pathogenic agents isolated from blood culture

b. Antimicrobial susceptibility profile of isolated bacteria (routine panel of antibiotics)

As part of this study we request that all isolates from blood will be stored and archived at –80ºC

These isolates will be re-cultured at a later date, the identification will be re-confirmed. Selected isolates will have:

i. Additional antimicrobial sensitivity testing

ii. Molecular analysis for antimicrobial resistance genes

iii. Genome sequencing of selected strains (defined by antimicrobial susceptibility data)

Laboratory methods

Blood sampling for culture and metabolomics

According to local procedures there are currently no systematic criteria concerning which patients should have a blood culture performed. However, generally in this setting a blood culture is indicated in neonates whose diagnosis is "probable sepsis". "Probable sepsis" is defined as having ≥2 clinical and ≥2 laboratory signs of sepsis as defined here. A

positive blood culture result provides a definitive diagnosis of sepsis and the isolation of a bacterium from a blood culture is the only method used in this location to confirm a diagnosis of sepsis. Empiric antimicrobial therapy is initiated while awaiting culture results and at least one blood culture is performed prior to initiating empirical antimicrobial therapy in neonates with "probable sepsis".

Documents/equipment/reagents to be used

2 printed study labels for blood samples

Blood Agar, MacConkey Agar or Sabouraud Dextrose Agar

Blood culture bottle: PED PLUS ™/F

BACTEC 9120, BACTEC FX TOP automated analyser

VITEK 2 COMPACT automated machine

Storage boxes and aliquots

Freezer –80^{o}C

Sampling

As a procedure in routine care, venous blood (1–2 mL) will be taken by experienced nurses after skin disinfection with povidone-iodine and alcohol. An additional 0.5–1 mL of blood will be taken in an EDTA tube and separated into cells and plasma, which will be stored at –80oC.

All bacteria isolated during the outlined period of analysis will be studied for their antimicrobial susceptibly profiles and the presence/absence of antimicrobial resistance genes.

Blood Culture Protocol

a) Receive the blood sample (1–2mL of venous blood injected into the Ped Plus ™/F bottle) from the clinical department and the sample request form. Check the information written on the Ped Plus ™/F bottle and the indication order.

b) Put the Ped Plus TM/F bottle into the BACTEC automatic blood culture systems

- BACTEC 9120
- BACTEC FX Top
- Automatically checked every 10 minutes
- Alarm sounds if suspected positive blood culture

c) If the blood culture is flagged as a suspected positive by the analyser:

- Take 1 mL of broth out of the Ped Plus™/F bottle to make a smear for the Gram-staining and bacterial subculture
- If Gram-stain result shows bacteria, they are identified as Gram-positive, Gram-negative, cocci, bacilli or fungi and the clinicians will be informed immediately by telephone.
- Sub-culture the broth onto 5% Blood Agar with a *S. aureus* "Satellite line" and chocolate agar and incubate at 35–37ºC in 5% CO_2 incubator and MacConkey Agar incubated at 35–37ºC in air for 24–48 hours. If the Gram stain shows suspected fungi a Sabouraud Dextrose Agar will be inoculated as well and incubated for 5 days. Return the Ped Plus ™/F bottle back into the automated analyser.

d) Isolation of bacteria or fungi

- After 24–48 hours, observe for the appearance of bacterial or fungal colonies
  - If bacterial colonies appear, identification and antimicrobial susceptibility tests will be performed using the VITEK 2 Compact.

- Organisms not identified by the VITEK 2 will be identified by API identification kits (Bio-Mérieux, Craponne, France) or specific molecular testing (16s RNA) if still not identified.
- If bacterial colonies do not appear, continue to follow-up the blood culture bottle in the BACTEC automatic blood culture systems.
- If the blood culture bottle is negative after 5 days of incubation then the result is given as “negative after 5 days”.

e) Interpretation of blood culture results

- The results of positive blood cultures are reported to the clinicians as soon as they are available. Discuss with the clinical staff to check if the blood culture results are consistent with the clinical signs and symptoms or whether the isolate is likely to be a contaminant.

- If the blood culture bottle grows two bacteria or more this may suggest contamination. The clinical significance of the result should be discussed with the clinical team
- Negative blood culture: The result is reported as “negative after 7 days”.

Antimicrobial susceptibility testing

When required for checking or to non-routine antimicrobials, antimicrobial susceptibility testing of the pathogens isolated will be performed by the disk diffusion method using guidelines established by the Clinical and Laboratory Standards Institute (CLSI) and, when required, by minimum inhibitory concentrations (MICs) by VITEK 2 COMPACT automated machine.

We will aim to test antimicrobial susceptibilities against nalidixic acid, ciprofloxacin, pefloxacin, ceftriaxone, cefotaxime, ceftazidime, cefepime, ampicillin, trimethoprim-sulfamethoxazole, azithromycin, chloramphenicol, imipenem, meropenem, ertapenem, colistin and amikacin for all Gram-negative organisms as well as oxacillin and vancomycin in Gram-positive organisms.

The production of extended-spectrum β-lactamases (ESBL) will be investigated using the double-disc synergy test by comparing zone sizes between ceftazidime discs against ceftazidime-clavulanic acid discs and cefotaxime discs against cefotaxime-clavulanic acid discs. Isolates with an increase in diameter of inhibitory zone of equal to or more than 5 mm by the synergy of clavulanate will be considered ESBL positive.

Bacterial storage

a) Storage of the bacterial isolates must be performed by trained laboratory staff of OUCRU.

b) Subculture the organisms onto 5% blood agar and ensure the purity of the isolate before storage.

c) Using Glycerol 20% as the storage media.

d) Transfer the culture to a storage tube equipped with a screw cap.

e) Put storage tubes into storage boxes and record the position in the register

f) Transfer the storage boxes to -80^0C freezer for long-term storage for 2–3 years.

Blood cells/plasma storage

a) Additionally, blood will be separated into cells and plasma by centrifugation.

b) Plasma and cells will be aliquoted into separate tubes equipped with a screw cap, labelled and stored to -80^0C freezer for long-term storage for 2–3 years

c) Samples will be shipped to Umea University in Sweden for metabolomic profiling.

Isolation of nucleic acids

At a later date isolates will be re-cultured and their identification re-checked

DNA will be extracted from bacterial isolates using the Wizard Genomic DNA Extraction Kit (Promega, Fitchburg, USA). This is a standard method in the laboratories at OUCRU-VN.

The quality and concentration of the DNA will be assessed using a nano-drop spectrophotometer prior to PCR amplification and the Quant-IT Kit (Invitrogen, Carlsbad, CA) prior to DNA sequencing.

PCR for resistance genes

The primary focus study is to investigate the distribution of antimicrobial resistance genes in bacteria causing neonatal BSIs at CH1. Therefore, all Gram-negative organisms will be investigated by PCR to detect genes catalysing resistance to cephalosporins, fluoroquinolones and carbapenems.

Conventional PCR will be performed for the following classes of resistance genes using previously described methods. The multiplex and monoplex PCRs are described in these publications (131,255–260).

This panel of PCRs will be used; PCR1 - AmpC (MOX-1, MOX-2, CMY-1, CMY8-11), PCR2 - AmpC (LAT-1 to LAT-4, CMY2-7, BIL-1), PCR3 - AmpC (DHA1 and DHA-2),

PCR4 - AmpC (ACC), PCR5 - AmpC (MIR-1T, ACT-1), PCR6 - AmpC (FOX-1-5b), PCR7 - ESBL (CTX-M1), PCR8 - ESBL (CTX-M2), PCR9 - ESBL (CTX-M9), PCR10 - ESBL (CTX-M8/M25), PCR11 - ESBL (TEM), PCR12 - ESBL (SHV), PCR13 - ESBL (OXA1, 4, 30), PCR13 - qnrA, B, S, and PCR13 - gyrA, B, C, PCR14 - NDM-1, PCR 15 - mecA/Van.

Genome sequencing

Selected organisms will be genome sequenced and on the basis of their susceptibility profiles and resistance gene content. Therefore, we cannot currently predict how many genomes will be sequenced. We aim to sequence the greatest cross-section of organism groups as possible.

Selected bacterial isolates will be sequenced at OUCRU-VN or at one of our collaborating genome sequences institutions, should facilities be available. Briefly, index-tagged paired end Illumina sequencing libraries will be prepared using one of 96 unique indexing tags as previously described. These will be combined into pools of uniquely tagged libraries and sequenced on the Illumina Genome Analyzer GAII or HiSeq sequencer according to manufacturer's protocols to generate tagged 54-100 bp paired-end reads. This is a previously described for Gram-negative organisms and Staphylococcus (137,246,261).

Analysis plan

Statistical comparisons

Data will be presented in the form of tables and bar charts for descriptive variables i.e. number of specific organisms per year and number of resistant organisms per year.

The proportion of cultured isolates by month, the antimicrobial susceptibility patterns will be determined. These data will be placed in the context of the broader CH1 population, by comparison of these data with historical laboratory records of pathogens isolated from patients with blood stream infections. Historical data from both neonates and older children will be analysed descriptively, and where appropriate, time trend analyses will be performed to determine significant alterations in bloodstream infection aetiology.

All statistical analysis will be performed using Stata version 11 (Stata Corp LP, College Station, TX, USA) and R; and p-values of ≤ 0.05 will be considered significant.

Antimicrobial resistance genes

The presence/absence of antimicrobial resistance genes will be reported as proportions per organism and then stratified by organism, year, and hospital ward.

Genome sequencing

Genome sequences will be analysed to study phylogenetic relationships, the presence/absence of genes and also antimicrobial resistance gene content and firstly analysed by species and then a group of Gram-negative organisms. Briefly, for phylogenetic analysis, chromosomal Single Nucleotide Polymorphism (SNP) alleles will be concatenated for each strain to generate a multiple alignment of all SNPs. For maximum likelihood (ML) analysis, RAxML will be run 10 times using the generalized time-reversible model and one thousand bootstrap pseudo-replicate analyses were performed to assess support for the ML phylogeny. Root-to-tip branches will be extracted from the ML tree using the program TreeStat. The relationship between root-to-tip distances and year of isolation were analysed using linear regression. For BEAST analysis

(v1.6), a GTR+Γ substitution model and defined tip dates, as the date of isolation will be used (137,246,261).

To detect the presence or absence of genes read sets will be assembled using the de novo short read assembler Velvet and Velvet Optimizer. Strain specific read sets will then be aligned to the pan-genome. Taxonomic investigation of accessory and resistance genes will be performed using MG-RAST v3.2 (http://metagenomics.anl.gov).

Metabolic profiling

Metabolomic profiling of plasma samples will be performed at Umea university in Sweden. Samples will be shipped frozen, processed and Chromatograms/mass spectra of the samples will be generated and analysed by blinded operator in a random order using Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry (GCxGC-TOFMS) and/or High Throughput Ultra-Performance Liquid Chromatography/Quadrupole-Time-of-Flight Mass Spectrometry (UPLC-Q-TOFMS). Acquired and processed data will be analysed by infecting bacterial species and AMR profiles using chemometrics based pattern recognition.

Data management

Data collection and entry

1. The investigator is responsible for maintaining all study records. The investigator is responsible for the timeliness, completeness and accuracy of the information in the original dataset and the clinical data management system.

2. CH1/OUCRU-VN staff will enter data directly onto computer-based data collection programs which will be uploaded securely to an Internet-based database.

3. Laboratory staff will record specimens (and their aliquots), their storage location, their shipments using a central commercial database system (Freezerworks).

4. All necessary tools, instruction, and training will be provided to all site staff involved in data entry to ensure the correct and consistent completion database prior to the study starting.

Record Retention

The investigator is responsible for retaining all essential data for at least 15 years after the completion of the study. Original paper documents will be maintained for a minimum of 5 years and electronic documents retained thereafter. All stored records are to be kept secure and confidential.

Quality control and quality assurance procedures

The study will be conducted in compliance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. Regular monitoring will be performed according to ICH GCP. Data, samples and procedures will be evaluated for compliance with the protocol, standard operating procedures, regulatory requirements and terms of ethical approval. Records will be verified for accuracy against source documents and physical inventory of samples. The diagnostic laboratory at CH1 conducts regular internal and external quality control procedures.

Ethics

Ethical and Regulatory Guidelines

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki (Seoul 2008) and the terms of approval of the appropriate ethical committees.

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice July 1996.

Ethical Review

This protocol and the relevant supporting document will be submitted to the EC/IRB of CH1 for review and will not be initiated at that site until after approval. The Investigators will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Informed Consent

Written informed consent will be obtained from all study participants or their representatives before any data from patients is collected for the study.

The study staff will discuss the study with all parent/representative of potential participants. Study staff will describe the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of patient, and alternatives to enrolment. The parent/representative will be invited to ask questions which will be answered by study staff, and they will be provided with appropriate numbers to contact if they have any questions subsequently. If the parent/representative agrees for their child to participate, they will be asked to sign and date two copies of an informed consent form. A copy of the form will be given to them to keep. If required, the parent/representative will be given up to 48 hours to consider for their children to take part in the study.

In addition to the procedures above, illiterate signatories will have the informed consent form read to them in the presence of a witness who will sign to confirm that the form was read accurately and that the participant or representative agrees to participation. All informed consent forms will be written in the local language and will use terms that are easily understandable.

Risks and benefits

This is a minimal risk study because it does not involve any investigational new drugs or interventions. The collection of all biological samples for use in this study have been performed as part of routine clinical assessments and are consistent with the local standard of care and good clinical practice. By being in the study, the patient will have access to experienced doctors in the hospital, who will examine and give advice at no cost at all visits and we also aim to find the cause of the sepsis so that the patient receives the correct medication.

Participant Expenses

Patients do not have to pay any expenses to participate in this study. Patients will not receive reimbursement of expenses.

Participant Confidentiality

The study staff will ensure that:

1. All data will be stored securely at the study site in locked file cabinets or password protected devices in areas with access limited to study staff.

2. All specimens, reports, study data collection, process, and administrative forms will be identified by a coded number.

3. Study databases will be secured with password-protected access systems and controlled distribution web-based security certificates.

4. No patient identifying information will be included in publications or presentations resulting from this work.

Sample Use and Storage

Use of stored human specimens

Blood cells and plasma samples will be stored at CH1 before shipping to Sweden for metabolite profiling, no sampled will be stored long-term beyond the duration of the project.

Sample shipment and overseas investigations

Investigations in part of this study may take part at CH1, OUCRU-VN or other collaborating institutions internationally i.e. samples will be required to be sent overseas for laboratory analysis outside current laboratory capabilities of CH1 and OUCRU-VN to the collaborating institutions. All investigators will be informed on any material leaving CH1/OUCRU-VN and appropriate requests to regulatory authorities will be made.

The current locations identified for collaboration include; the Wellcome Trust Sanger Institute (Hinxton, United Kingdom), the University of Sydney (Australia), the University of Melbourne (Australia) and the University of Umea, Sweden. The material sent overseas for this study will constitute the primary incidence nucleic acid for DNA sequencing only as CH1/OURCU-VN currently does not have the capacity for large-scale bacterial genome sequencing.

Additionally, plasma and blood cells will be shipped to Umea University in Sweden for metabolomic profiling, this technology does not currently exist in Vietnam and Professor Baker has a long-standing collaboration with this group. This material will be sent under MOU's and collaboration agreement between OUCRU-VN and these institutions.

CH1/OUCRU-VN will remain the owners of all sequence/metabolomic data and will oversee all analyses. Therefore, collaborations to achieve the aims of this protocol are essential. Depending on the genomic sequences, bacteria may also need to be shipped for secondary analysis or phenotyping, as CH1/OURCU-VN currently does not have the capacity for high-throughput phenotyping. For other studies outside this protocol, additional IRB approval will be sought.

Long-term storage of bacteria specimens

The CH1 microbiology laboratory will hold and store the samples for this study. Nucleic acid extractions will be stored until all analyses for this study have been performed. Blood cells and plasma will not be stored beyond the duration of the project.

Future investigations at CH1/OUCRU-VN

Additional investigations may be performed on isolated nucleic acids and bacteria collected as part of this study. These samples will only be used to study the aetiology, the epidemiology, or the phenotype of these organisms and will occur in the CH1 microbiology laboratory, the OUCRU-VN laboratory of Prof. Stephen Baker and external laboratories (with permission from all collaborating institutions). The principal investigators will make decisions on these studies. For other studies outside this protocol, additional IRB approval will be sought.

Finance and insurance

The conduct of this study is mainly funded by the Royal Society and the Wellcome Trust of the United Kingdom and sponsored by the University of Oxford. The University has a specialist insurance policy in place, Newline Underwriting Management Ltd, at Lloyd's of London, which would operate in the event of any participant suffering harm as a result of their involvement in the research.

Publication plan

As this is a CH1/OUCRU-VN collaboration, the contributions of both CH1 and OUCRU investigators will both be recognized in authorship allocation. The authors (and their respective positions in the author list) will be agreed prior to the start of the study in accordance with the guidelines of the International Committee of Medical Journal Editors.

Data from this study is of substantial interest to the scientific and clinical research communities. In line with Wellcome Trust policy that the results of publicly-funded research should be freely available, manuscripts arising from this study will, wherever possible, be submitted to peer-reviewed journals which enable Open Access via UK PubMed Central (PMC) within six months of the official date of final publication. All publications will acknowledge the trial's funding sources.

In line with research transparency and greater access to data OUCRU's data sharing policy will be implemented. This policy is based on a controlled access approach with a restriction on data release that would compromise an ongoing trial or study.

Data exchange complies with Information Governance and Data Security Policies in all

of the relevant countries.

9.5 The participant's information sheet

Children's Hospital 1

Oxford University Clinical Research Unit

Participant's Information Sheet for the study

"Bloodstream infections of new-born babies"

Information about this study

We are inviting parents/guardians of children attending this hospital with the diagnosis of sepsis to participate in a research study called "The clinical features, antimicrobial susceptibility patterns and genomics of bacteria in neonatal sepsis". The aims of this research study are to identify the clinical features and causes of sepsis in an effort to treat your child with the correct medication. We are also interested in gathering information to understand about the antimicrobial susceptibility, the genes of bacteria causing sepsis and the impact of sepsis on the disease.

This research is being done by the Oxford University Clinical Research Unit and the Children's Hospital 1 and has been approved by the ethical committee of both institutions. This document describes your rights, what will happen during the study, the benefits and risks so that you have all the appropriate information to decide about whether to enrol your child in the study. If there is any information that you do not understand, please ask one of the study staff. All of your questions will be answered.

If I consent on behalf of my child, what will happen to my child and me in this study?

Whether or not you and your child join the study, your child will receive the standard treatment for his/her current diseases. If you agree for your child to participate in this study, your child's basic clinical and demographic data will be collected. As routine care, he/she will be collected blood sample for microbiology tests for the diagnosis and treatment of your child. All of this happens in the laboratory and does not involve you in any way. The results will be reported to your doctor according to standard hospital procedures. The study team will record data about your diagnosis, treatment and hospital stay, but will do so anonymously.

Risks and benefits to being in the study

This is no risk in participating in this study because it does not involve in testing any new drugs or interventions. All tests have been performed as part of routine clinical assessments and are consistent with the standard of care at Children's Hospital 1. We also aim to find the cause of the sepsis so that the patient receives the correct medication.

Confidentiality

All of the information we get from you is strictly private. Your child's name will not be on any samples we collect or test results–we will use a number instead of a name. Your name, or your child's name, will not be mentioned in any output from this study. We will not use your information for any purpose outside this study or give it to anyone else. Any information obtained from any individual in connection with this study will be kept strictly confidential.

Costs

The Oxford University Clinical Research Unit is giving funding for this program. There will be no cost to you for participating in this study. The program does not pay for any specific treatment or hospital costs and you will be responsible for paying these as you usually would.

Voluntary participation

If you do not want to be part of this study, it will not affect the care your child will receive in any way. Even if you do agree to become a study participant, you can withdraw from the study at any time (verbally) without affecting the care that you or your child will receive. If you decide at any time to take your child out of the program, no new information will be collected. However, information collected on your child up until that point will still be used.

Obtaining additional information

You are encouraged to ask any questions related to this study during the time of participation. If you have any questions about this program, its procedures, risks and benefits, or alternatives please call Dr. Nguyen Duc Toan at 090 253 8684.

If you have questions about being a participant in a research study please contact the Research Ethics Board of the Children's Hospital 1 at 08 3927 1119 – 282 or the Research Office at the OUCRU at 08 3923 1983.

9.6 Consent form

Informed Consent Form

for the study

"Bloodstream infections of new-born babies"

(to be signed by the participant's parent or guardian)

I have read the information given to me and freely agree for my child to be in this study. I will be given a copy of this form to keep.

I have been told about the risks and benefits. I got answers that I could understand to all my questions.

I understand that my child may stop participating in the study at any time. If my child stops the study, it will not affect he/she medical treatments. I consent that information collected up until that point will still be used.

Participant Number: 19EN – ____ ____ ____

Participant's name: _____

By signing/marking my name here, I confirm what is written above.

_____	_____
Signature of person giving consent	**Relationship to participant**
_____	_____
Print name	**Date of signature**

Confirmation of study staff:

I, the undersigned, have fully explained the relevant information of this study to the participant named above and will provide her/him with a copy of this signed and dated informed consent form. If the subject is over 12 years old, I confirm they have given verbal assent. The participant voluntarily agrees to be in the study.

Investigator / designee signature	**Print name**	**Date of signature**

If the person giving consent cannot read the form, a witness must be present and sign here:

I was present throughout the entire informed consent process with the participant. All questions from the participant were answered and the participant has agreed to take part in the research.

Witness signature	**Print name**	**Date of signature**

9.7 Case report form

Patient data of my study were collected on individual case report forms.

19EN - Neonatal Sepsis

FEATURE			**FEATURE (1 / 2)**
Study Identifier **[19EN]**	Study Site Identifier **[001]**	Subject Identifier [__|__|__|__|__|__]	Subject Initials [__|__|__|__|__|__]

ADMINISTRATION INFORMATION

1. Hospital Number: [__|__|__|__|__|__|__|__|/|__|__]
2. Sex: ○ Male ○ Female
3. Residence: [_____]
4. Father's job: [_____]
5. Mother's job: [_____]
6. Admitted from: ○ Home ○ Transferred from hospital [_____]
   Treated From: [__|__]/[__|__]/[__|__] (dd/mm/yy)
   To: [__|__]/[__|__]/[__|__] (dd/mm/yy)
7. Date of CH1 admission: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)
8. Date of CH1discharge: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)

DELIVERY INFORMATION

9. Hospital of birth: [_____]
10. Date of birth: [__|__]/[__|__]/[__|__] (dd/mm/yy)
    Time: [__|__]:[__|__] (hh:mm) Unknown ☐
11. Gestational age: [__|__] weeks Unknown ☐
12. Birth weight: [__|__|__|__] grams Unknown ☐
13. This birth occurred outside hospital ○ Yes ○ No
    If Yes ○ Home birth ○ Birthing center ○ En route to hospital
    ○ Unknown ○ Other location , specify: [_____]
14. Type of delivery: ○ Vaginal ○ C-section ○ Forceps ○ Vacuum ○ Unknown
15. Apgar score at 1/5 minute: [__|__]/ [__|__] Unknown ☐
16. Perinatal asphyxia/Hypoxic ischemic encephalopathy: ○ Yes ○ No ○ Unknown
17. Resuscitation/invasive procedures at delivery room: ○ Yes ○ No ○ Unknown
18. Intrapartum fever (> 38oC): ○ Yes ○ No ○ Unknown
19. Suspected/proven chorioamnionitis: ○ Yes ○ No ○ Unknown
20. Duration of membrane rupture >18 hours: ○ Yes ○ No ○ Unknown
21. Maternal hot bed: ○ Yes ○ No ○ Unknown
22. Other delivery information, specify: [_____]

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FEATURE	FEATURE (2 / 2)

CLINICAL SIGNS OF SEPSIS

23. Fever: ○ Yes ○ No ○ Unknown
24. Highest temperature: [__|__] . [__] oC
25. Hypothermia: ○ Yes ○ No ○ Unknown
26. Lowest temperature: [__|__] . [__] oC
27. Temperature instability: ○ Yes ○ No ○ Unknown
28. Bradycardia: ○ Yes ○ No ○ Unknown
29. Lowest heart rate: [__|__|__] beats/min
30. Tachycardia: ○ Yes ○ No ○ Unknown
31. Highest heart rate: [__|__|__] beats/min
32. Rhythm instability: ○ Yes ○ No ○ Unknown
33. Reduced urinary output: ○ Yes ○ No ○ Unknown [__|__|__] ml/day [__] . [__] ml/kg/day
34. Hypotension ○ Yes ○ No ○ Unknown MAP: [__|__] mmHg
35. Mottled skin: ○ Yes ○ No ○ Unknown
36. Impaired peripheral perfusion: ○ Yes ○ No ○ Unknown
37. Apnea episodes: ○ Yes ○ No ○ Unknown
38. Slow breathing episodes: ○ Yes ○ No ○ Unknown
39. Lowest respiratory rate: [__|__] breaths/min
40. Tachypnea episodes: ○ Yes ○ No ○ Unknown
41. Highest respiratory rate: [__|__] breaths/min
42. Increased oxygen requirements: ○ Yes ○ No ○ Unknown
43. Requirement for ventilator: ○ Yes ○ No ○ Unknown
44. Poor sucking: ○ Yes ○ No ○ Unknown
45. Prolonged feeding intolerance: ○ Yes ○ No ○ Unknown
46. Abdominal distension: ○ Yes ○ No ○ Unknown
47. Petechial rash: ○ Yes ○ No ○ Unknown
48. Sclerema: ○ Yes ○ No ○ Unknown
49. Irritability: ○ Yes ○ No ○ Unknown
50. Lethargy: ○ Yes ○ No ○ Unknown
51. Altered mentation: ○ Yes ○ No ○ Unknown
52. Hypotonia: ○ Yes ○ No ○ Unknown
53. Severe jaundice ○ Yes ○ No ○ Unknown
54. Seizures ○ Yes ○ No ○ Unknown
55. Other features (if any) [_____]

LABORATORY **LAB (1 / 1)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[19EN]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

LABORATORY SIGNS OF SEPSIS

1. WBC min: [__|__] . [__] x $10^3/mm^3$
2. WBC max: [__|__] . [__] x $10^3/mm^3$
3. ANC min: [__|__] . [__] x $10^3/mm^3$
4. ANC max: [__|__] . [__] x $10^3/mm^3$
5. I/T: [__] . [__|__]
6. Band Neutrophil: [__|__] . [__] %
7. Platelet Count: [__|__|__] . [__] x $10^3/mm^3$
8. CRP: [__|__|__] . [__|__] mg/L
9. Procalcitonin: [__|__] . [__|__] ng/mL
10. Blood Glucose Min: [__|__|__] . [__] mg/dL
11. Blood Glucose Max: [__|__|__] . [__] mg/dL
12. Metabolic acidosis ○ Yes ○ No ○ Unknown
    If Yes, Base excess: - [__|__] . [__] mEq/L
13. PaO_2/FiO_2: [__|__|__] mmHg
14. Lactate: [__|__] . [__] mmol/L

ORGAN DYSFUNCTION

15. Total Bilirubin: [__|__|__] . [__] µmol/L
16. Serum Creatinine: [__|__|__] . [__] µmol/L
17. AST [__|__|__|__] IU/L
18. ALT: [__|__|__|__] IU/L
19. Hemoglobin Min: [__|__] . [__] g/dL
20. Hemoglobin max: [__|__] . [__] g/dL
21. Electrolyte disturbance: ○ Yes ○ No ○ Unknown
22. Abnormal coagulation/DIC: ○ Yes ○ No ○ Unknown
23. Heart failure/ cardiac injury: ○ Yes ○ No ○ Unknown
24. Brain lesions: ○ Yes ○ No ○ Unknown
    If Yes, specify: [_____]
25. Eye problems: ○ Yes ○ No ○ Unknown
    If Yes, specify: [_____]
26. Organ dysfunction ○ Yes ○ No ○ Unknown
    If Yes, Free from organ dysfunction: [__|__|__] days
    Number of dysfunctional organs: [__|__]

DIAGNOSIS **DIAG (1 / 1)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[19EN]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

1. Discharge diagnoses: [_____]

ICD-10 code 1: [_____]
ICD-10 code 2: [_____]
ICD-10 code 3: [_____]
ICD-10 code 4: [_____]

2. Probable sepsis: ○ Yes ○ No ○ Unknown
Diagnostic date: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)

3. Confirmed sepsis: ○ Yes ○ No ○ Unknown
Diagnosis date: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)

4. Severe sepsis: ○ Yes ○ No ○ Unknown

5. Septic shock ○ Yes ○ No ○ Unknown

6. Onset of sepsis: ○ Early ○ Late ○ Unknown
Diagnosis date: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)

7. Acquisition of sepsis: ○ Community ○ Hospital ○ Healthcare ○ Unknown
Diagnosis date: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)

8. Sequences of sepsis: [__|__] episode(s)

9. Infection syndromes:

Asymptomatic bacteremia	☐	Cellulitis	☐
Meningitis/encephalitis	☐	Dermal infection	☐
Pneumonia	☐	Central line related sepsis	☐
Urinary infection	☐	HIV infection ☐ , specify:	[_____]
Peritonitis	☐	Tuberculosis ☐	
Necrotizing enterocolitis	☐	TORCH ☐ , specify:	[_____]
Umbilical infection	☐	Unknown ☐	

Others: [_____]

10. Associated diagnoses:

Congenital heart diseases	☐	Congenital renal diseases	☐
Broncho-pulmonary dysplasia	☐	Neuromuscular diseases	☐
Pulmonary hypertension	☐	Congenital GI anomalies	☐
RDS	☐		

Others: [_____]

MANAGEMENT **MGMT (1 / 1)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[19EN]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

1. Empirical antibiotics: ○ Appropriate ○ Inappropriate ○ Unknown

Antibiotics use:

Antibiotic	If other *	Used	Dose	Duration	As
Amikacin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Amphotericin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Ampicillin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Cefepime	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Cefotaxime	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Ceftazidime	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Ciprofloxacin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Colistin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Gentamicin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Imipenem	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Meropenem	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Metronidazole	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Oxacillin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Pefloxacin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Ticarcillin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Vancomycin	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Other 1	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line
Other 2	[_____]	☐	[__|__|__|__]mg/kg/day	[__|__]days	[__|__]line

* *Only enter for Other*

2. Duration of intubation/ventilator support: [__|__|__] days
3. Duration of total parenteral nutrition: [__|__|__] days
4. Duration of in-travenous lipid emulsion: [__|__|__] days
5. Duration of central vascular catheters: [__|__|__] days
6. Invasive surgical or procedural intervention ☐ Specify: [_____]
7. Shock management: ○ Yes ○ No ○ Unknown
   Dopamine: [__|__] Dobutamine: [__|__] Epinephrine: [__] . [__] (µg/kg/min)
8. Blood products transfusion: ○ Yes ○ No ○ Unknown
9. Antacid agents: ○ Yes ○ No ○ Unknown
10. Corticosteroids/immunosuppression agents: ○ Yes ○ No ○ Unknown
11. Other drugs ○ Yes ○ No ○ Unknown If Yes, specify: [_____]
12. Score of severity (at most severe day of sepsis): [__|__|__]

OUTCOME			**OUTCOME (1 / 1)**
Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[19EN]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

Department	**From**		**To**	
[______________]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]
[______________]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]
[______________]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]
[______________]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]
[______________]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]
[______________]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]

OUTCOME OF HOSPITALIZATION

At	**Outcome ***	**Department**	**If other, Specify**
Day 7 after admission	[____]	○ ND ○ NICU ○ Other dept	[____________]
Day 14 after admission	[____]	○ ND ○ NICU ○ Other dept	[____________]
Day 21 after admission	[____]	○ ND ○ NICU ○ Other dept	[____________]
Day 28 after admission	[____]	○ ND ○ NICU ○ Other dept	[____________]

** 1: Survival; 2: Survival and Organ failure; 3: Death; 4: Death and Organ failure*

- Septic outcome of hospitalization:
  - Infectious complications ☐
  - Polymicrobial bacteremia ☐
  - Sepsis attributable death ☐

- Overall outcome of hospitalization: ○ Discharge ○ Death ○ Home to die [________________]

TOTAL COST OF TREATMENT: [__|__|__|__|__|__|__|__|__] **VNĐ** **COST OF ANTIBIOTICS:** [__|__|__|__|__|__|__|__|__] **VNĐ**

MICROBIOLOGY **MICRO (1 / 2)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[19EN]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

ISOLATED ORGANISM # **[__|__]**

Sample: ○ Blood ○ CSF ○ NTA/ETA ○ Urine ○ Stool ○ Other [_____]

Sample code: **[_____]**

Date of sampling: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)

Date of delivery: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)

Date of results: [__|__]/[__|__]/[__|__] (dd/mm/yy) Time: [__|__]:[__|__] (hh:mm)

Clinical department where the organism is isolated:
○ Emergency
○ Neonatology
○ Neonatal ICU
○ Other , specify: [_____]

Organism:

Acinetobacter spp	☐	Klebsiella spp	☐
Acinetobacter baumannii	☐	Klebsiella pneumoniae	☐
Acinetobacter [_____]		Klebsiella [_____]	
Burkholderia spp	☐	Morganella spp	☐
Burkholderia cepacia	☐	Morganella morganii	☐
Burkholderia [_____]		Morganella [_____]	
Candida spp	☐	Proteus spp	☐
Candida albicans	☐	Proteus mirabilis	☐
Candida [_____]		Proteus [_____]	
Coagulase-negative Staphylococcus	☐	Pseudomonas spp	☐
Escherichia coli	☐	Pseudomonas aeruginosa	☐
Enterobacter spp	☐	Pseudomonas [_____]	
Enterobacter cloacae	☐	Staphylococcus aureus	☐
Enterobacter [_____]		Streptococcus spp	☐
Enterococcus spp	☐	Streptococcus agalactiae	☐
Enterococcus faecalis	☐	Streptococcus [_____]	
Enterococcus [_____]		Other [_____]	

ESBL ○ Positive ○ Negative ○ Not Done

MIC value (Vancomycin): [__|__] . [__|__] µg/mL

MICROBIOLOGY **MICRO (2 / 2)**

Antimicrobial susceptibility patterns

Amikacin	○ S ○ I ○ R
Amphotericin B	○ S ○ I ○ R
Ampicillin	○ S ○ I ○ R
Cefotaxime	○ S ○ I ○ R
Ceftazidime	○ S ○ I ○ R
Cefuroxime	○ S ○ I ○ R
Cefoperazone	○ S ○ I ○ R
Cefepime	○ S ○ I ○ R
Chloramphenicol	○ S ○ I ○ R
Ciprofloxacin	○ S ○ I ○ R
Co-trimoxazole	○ S ○ I ○ R
Erythromycin	○ S ○ I ○ R
Ertapenem	○ S ○ I ○ R
Gentamicin	○ S ○ I ○ R
Imipenem	○ S ○ I ○ R
Meropenem	○ S ○ I ○ R
Metronidazole	○ S ○ I ○ R
Nalidixic acid	○ S ○ I ○ R
Nitrofurantoin	○ S ○ I ○ R
Norfloxacin	○ S ○ I ○ R
Oxacillin	○ S ○ I ○ R
Pefloxacin	○ S ○ I ○ R
Penicillin	○ S ○ I ○ R
Polymycin B	○ S ○ I ○ R
Rifampicin	○ S ○ I ○ R
Ticarcillin	○ S ○ I ○ R
Vancomycin	○ S ○ I ○ R
[_____]	○ S ○ I ○ R
[_____]	○ S ○ I ○ R
[_____]	○ S ○ I ○ R

9.8 The Neonatal Therapeutic Intervention Scoring System (NTISS)

In my study, the NTISS was used to evaluate the disease severity of patients with sepsis.

NTISS items	Sub-score*
Respiratory	
Surfactant administration	1
Endotracheal intubation	2
Extracorporeal membrane oxygenation	4
Supplemental oxygen	1a
Continuous positive airway pressure administration	2a
Mechanical ventilation	3a
Mechanical ventilation with muscle relaxation	4a
High-frequency ventilation	4a
Tracheostomy care	1b
Tracheostomy placement	1b
Cardiovascular	
Ibuprofen administration	1
Cardiopulmonary resuscitation	4
Volume expansion (≤15 mL/kg)	1c
Volume expansion (>15 mL/kg)	3c
Vasopressor administration (1 agent)	2d
Vasopressor administration (>1 agent)	3d
Pacemaker on standby	3e
Pacemaker used	4e
Drug therapy	
Steroid administration (postnatal)	1
Anticonvulsant administration	1
Aminophylline administration	1
Other unscheduled medication	1
Treatment of metabolic acidosis	3
Potassium binding resin administration	3
Antibiotic administration (≤2 agents)	1f
Antibiotic administration (>2 agents)	2f
Diuretic administration (enteral)	1g
Diuretic administration (parenteral)	2g
Monitoring	
Frequent vital signs	1
Cardiorespiratory monitoring	1
Thermoregulated environment	1
Non-invasive oxygen monitoring	1
Arterial pressure monitoring	1
Central venous pressure monitoring	1
Urinary catheter	1
Quantitative intake and output	1
Phlebotomy (5–10 blood draws)	1h
Extensive phlebotomy (>10 blood draws)	2h
Metabolic/nutrition	
Gavage feeding	1
Intravenous fat emulsion	1

NTISS items (continued)	Sub-score*
Intravenous amino acid solution	1
Phototherapy	1
Insulin administration	2
Potassium infusion	3
Transfusion	
Intravenous gamma globulin	1
Partial volume exchange transfusion	2
Double volume exchange transfusion	3
Platelet transfusion	3
White blood cell transfusion	3
Red blood cell transfusion (≤15 mL/kg)	2i
Red blood cell transfusion (>15 mL/kg)	3i
Procedures	
Transport of patient	2
Thoracentesis	3
Dialysis	4
Single chest tube in place	2j
Multiple chest tubes in place	3j
Minor operation	2k
Major operation	4k
Pericardiocentesis	4l
Pericardial tube in place	4l
Vascular access	
Peripheral intravenous line	1
Central venous line	2
Arterial line	2

*Letters represent mutually exclusive parameters

Open Access Protocol

BMJ Open

Clinical features, antimicrobial susceptibility patterns and genomics of bacteria causing neonatal sepsis in a children's hospital in Vietnam: protocol for a prospective observational study

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ABSTRACT

Introduction The clinical syndrome of neonatal sepsis, comprising signs of infection, septic shock and organ dysfunction in infants ≤4 weeks of age, is a frequent sequel to bloodstream infection and mandates urgent antimicrobial therapy. Bacterial characterisation and antimicrobial susceptibility testing is vital for ensuring appropriate therapy, as high rates of antimicrobial resistance (AMR), especially in low-income and middle-income countries, may adversely affect outcome. Ho Chi Minh City (HCMC) in Vietnam is a rapidly expanding city in Southeast Asia with a current population of almost 8 million. There are limited contemporary data on the causes of neonatal sepsis in Vietnam, and we hypothesise that the emergence of multidrug resistant bacteria is an increasing problem for the appropriate management of sepsis cases. In this study, we aim to investigate the major causes of neonatal sepsis and assess disease outcomes by clinical features, antimicrobial susceptibility profiles and genome composition.

Method and analysis We will conduct a prospective observational study to characterise the clinical and microbiological features of neonatal sepsis in a major children's hospital in HCMC. All bacteria isolated from blood subjected to whole genome sequencing. We will compare clinical variables and outcomes between different bacterial species, genome composition and AMR gene content. AMR gene content will be assessed and stratified by species, years and contributing hospital departments. Genome sequences will be analysed to investigate phylogenetic relationships.

Ethics and dissemination The study will be conducted in accordance with the principles of the Declaration of Helsinki and the International Council on Harmonization Guidelines for Good Clinical Practice. Ethics approval has been provided by the Oxford Tropical Research Ethics Committee 35-16 and Vietnam Children's Hospital 1 Ethics Committee 73/GCN/BVND1. The findings will be disseminated at international conferences and peer-reviewed journals.

Strengths and limitations of this study

- Little is known about the current aetiological agents of neonatal sepsis in Vietnam. This prospective study will integrate clinical assessments with microbiological and detailed whole genome sequence data to characterise the aetiology and outcome of neonatal sepsis in this high-mortality setting.
- This study is being performed at the largest secondary/tertiary paediatrics centre in Southern Vietnam. Data collection at a single site may limit the applicability to other hospitals in the country.
- Other limitations encountered in designing this study include ethical issues involved in collecting samples from severely ill neonates, lack of current transferable definitions for neonatal sepsis phenotypes and stochastic variations in numbers of cases recruited due to seasonal variation and continuous changes in community antimicrobial and vaccine use.
- Some contamination of blood cultures is unavoidable in our setting and therefore accurately classifying some isolates as true pathogens in certain cases may be challenging.

Trial registration number ISRCTN69124914; Pre-results.

BACKGROUND

Neonatal sepsis is widely recognised as a clinical syndrome of systemic inflammation in response to, or occurring the same time as, a possible or proven infection (frequently by identifying bacterial bloodstream infection) occurring in children ≤28 days of age. A consensus definition of neonatal sepsis has remained a challenge.[1] Globally, the incidence

of neonatal sepsis is estimated to be 1–5 cases/1000 live births but is lower in full-term neonates (1–2 cases/1000 live births), in whom the incidence is higher in men than women.[2 3] Early-onset sepsis is defined as the start of sepsis symptoms within 72 hours of birth[1 4] and is often caused by vertical transmission of pathogens during delivery as a result of chorioamnionitis or maternal genital tract colonisation.[5] Late-onset sepsis, occurring after 72 hours from birth,[1 6] may be caused by similar vertical transmission or horizontal transmission mechanisms due to direct contact with the surrounding environment, attendant healthcare staff or any invasive procedures.[7]

Neonatal sepsis in Southeast Asia

Neonatal sepsis remains a leading cause of neonatal hospital admission, morbidity and mortality in low-income and middle-income countries (LMICs).[8] In this setting, bacterial infection, including bacteraemia, is complicated by multidrug resistance, particularly related to healthcare acquired infection, and effective management of neonatal sepsis is increasingly problematic.[8] Recently, WHO has acknowledged the problem of antimicrobial resistance (AMR) as an endemic and widespread problem in LMICs.[9] In many LMICs untreatable bacterial infections with broadly AMR pathogens are no longer a threat but a common reality. AMR in LMICs represents one of the biggest threats to global health and are one of the greatest current challenges in infectious disease research.

While AMR is an issue with all types of bacterial infection, the issue is most acute in management of clinical sepsis. This is a particular problem in neonates due to high mortality/morbidity rates and the timely need for rapid detection and treatment of the causative pathogen. Sepsis demonstrates extensive geographical diversity in both aetiology and proportions of AMR bacteria isolated.[10 11] Understanding the local and regional epidemiology of sepsis in hospitalised neonates is crucial in the development of rational management and treatment guidelines, especially in high-risk AMR LMICs locations like Vietnam.

Sepsis and AMR in Vietnam

Bacterial sepsis is classified into two major groups according to place of acquisition. Hospital-acquired sepsis is defined in patients with clinical manifestations of sepsis and a confirmatory blood culture collected >48 hours following hospital admission.[12] Hospital-acquired sepsis is a major threat to patient safety, and in locations with poor surveillance and infection control programme such infections are associated with high mortality rates. The incidence of AMR bloodstream infections in Vietnam has increased over recent years and is predicted to increase further.[13] This trend has comprised an increase in both Gram-negative and Gram-positive pathogens, chiefly *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus* and enterococci.

Community-acquired sepsis is also an important cause of fever among patients admitted to hospitals across South and Southeast Asia. In our practice, community-acquired sepsis is defined as the presence of sepsis with a confirmatory blood culture collected within 48 hours after hospital admission.[14] Distinguishing bacterial infections from other common causes of fever, such as malaria or dengue, can be challenging without diagnostic laboratory support.[15] Important causes of community-acquired bloodstream infection (CA-BSI) in LMICs include *Salmonella* serovars Typhi and Paratyphi A, *E. coli*, *K. pneumoniae*, *S. aureus* and *Streptococcus pneumoniae*. We have reported increasing levels of AMR in these common community-acquired pathogens and highlighted the difficulties of accurate diagnosis with traditionally available diagnostics. For example in Nepal and Vietnam antimicrobial resistance in *Salmonella* Typhi and *Salmonella* Paratyphi A has severely restricted the options available for antimicrobial treatment.[16]

Changing aetiology of BSI in Vietnam

The aetiology of CA-BSI in Vietnam has changed considerably over the 20 years. A previous study documented the decline of *Salmonella* Typhi from 2002, the predominant pathogen until this point and the subsequent increase in non-typhoidal *Salmonella* and other opportunistic HIV-associated pathogens.[17] This shift is likely to reflect a changing landscape of infectious disease related to the HIV epidemic, urbanisation and secondary social determinants within Vietnam. Vietnam, as with many countries in Asia, is undergoing a rapid economic transition, and programmes to improve sanitary conditions have reduced the overall risk of waterborne infections. HIV-associated opportunistic pathogens have now emerged as the leading cause of bloodstream infections and the primary cause of mortality in hospitalised adult patients in this location. These studies were performed at the Hospital for Tropical diseases in Ho Chi Minh City and thus included mainly adults, including those with HIV, and children but not neonates.[18–20] Therefore, these observations may not be fully representative of the situation in neonates.

Knowledge gaps

Little is known about contemporary antimicrobial susceptibility patterns and their underlying genetic determinants in the major causes of neonatal bacterial of sepsis in Vietnam. Furthermore, the impact of antimicrobial susceptibility and other virulence factors on disease progression and outcome in neonates in LMICs is also not well documented. To address these issues, we aim to investigate the aetiology of pathogens associated with bacterial sepsis in neonates and to detail the effects of reduced antimicrobial susceptibility on the outcome of sepsis in neonates. This will be a clinical and microbiology laboratory research project between Children's Hospital 1 and the Oxford University Clinical Research Unit (OUCRU) in Vietnam. This study will be a conduit for introducing molecular biology for bacteriology into routine hospital care at this children's hospital and will lead to future studies investigating appropriate empirical treatment for bacteraemia and the impact of antimicrobial

resistance on the outcome of sepsis in the paediatric and neonatal population in Vietnam.

Rationale, aim and objectives

To understand the causes of neonatal sepsis and to best inform antimicrobial treatment regimes in our setting, we will perform a prospective observational study at Children's Hospital 1 in Vietnam from 2017 to 2019. There are limited contemporary data on the causes of bacterial sepsis in neonates in Vietnam. We hypothesise that there have been recent increases in multidrug resistant Gram-negative bacteria causing sepsis in this high-risk group and that methicillin-resistant *S. aureus* has emerged as an important pathogen. We further aim to investigate the clinical features, major causes of neonatal sepsis and the distribution of pathogens by departments, their antimicrobial susceptibility patterns and the genomic profiles of the isolated bacteria as well as their association with disease outcomes.

Primary objectives

- To describe the clinical characteristics of neonates with sepsis, including community and hospital-acquired sepsis, early-onset and late-onset sepsis.
- To determine the aetiology of neonatal sepsis and the distribution of pathogens by clinical departments including the Neonatology Department and the Neonatal Intensive Care Unit.
- To determine the antimicrobial susceptibility profiles of the bacteria causing neonatal sepsis and the AMR profiles occurring in community and hospital-acquired infections.
- To analyse the impact of specific bacteria and AMR profile on the outcomes (mortality, length of stay and cost of treatment) of neonates with sepsis.
- To determine the genome sequences of bacterial strains associated with neonatal sepsis.

Secondary objectives

- To determine the AMR profiles and gene distribution of isolated bacteria by clinical departments to add insight into the circulation of bacteria associated with hospital acquired infections.
- To study the genes catalysing resistance to antimicrobials commonly used to treat neonatal sepsis (specifically third/fourth generation cephalosporins, fluoroquinolones and carbapenems).

METHODS

Study design

This protocol describes a prospective, non-interventional, observational study to characterise the clinical features of neonates with sepsis at Children's Hospital 1 in Ho Chi Minh City in Vietnam between 2017 and 2019, the microbial population structure, antimicrobial susceptibility patterns and the AMR genes of the bacteria causing that sepsis. All organisms isolated from blood will be stored and archived for molecular characterisation.

Study site

Children's Hospital 1 (Neonatology Department, Neonatal Intensive Care Unit, Microbiology Department) is in Ho Chi Minh City in Vietnam. The estimated population of the city was 8.4 million in 2016, and 23.8% are children 0–14 years of age.[21] Children's Hospital 1 is the largest tertiary paediatrics centre in Southern Vietnam with 1400 inpatient beds and >1600 staff members. The hospital receives ~1.5 million outpatient visits and 95 000 admissions each year. Care is provided to all children <15 years old from Ho Chi Minh City and other provinces of Southern Vietnam. The neonatal centre at this hospital currently has 120 inpatient beds for the neonatology department and additional 30 beds in the neonatal intensive care unit. The overall mean rate of positive blood cultures in our hospital is 7% per year.

DEFINITIONS

Definition of sepsis

A sepsis episode in this study is defined as isolation of a clinically relevant pathogen from ≥1 blood culture, drawn from a neonate with ≥1 clinical or laboratory sign of sepsis (table 1).[22]

Diagnosis of neonatal sepsis

Systematic guidelines concerning which patients should have blood cultures performed are not strictly defined in our hospital, although blood culture results are used to confirm the diagnosis of sepsis in neonates with a compatible clinical presentation. We use the criteria suggested by the European Medicines Agency in 2010 for the diagnosis of 'probable sepsis' and 'confirmed sepsis' in neonates[22]:

- Probable sepsis: ≥2 clinical and ≥2 laboratory signs;
- Confirmed sepsis: ≥1 positive culture of a pathogen and ≥1 clinical or laboratory sign.

SAMPLE SIZE

In this prospective observational study, we aim to recruit all patients with available data who fulfil the inclusion criteria and are admitted to Children's Hospital 1 in Ho Chi Minh City in Vietnam from 2017 to 2019. Based on retrospective surveillance data, we estimate recruitment of 800 participants during the study period. Blood cultures will be performed in all cases, we expect to yield ~400 bacterial isolates.

Participant selection and recruitment

Inclusion criteria

Neonates (≤1 month of age) with a diagnosis of 'probable' or 'confirmed' sepsis who have had a blood culture taken and who are an inpatient at Children's Hospital 1 will be recruited into the study after written informed consent has been given by a parent or guardian.

Exclusion criteria

Patients will be excluded when informed consent is not provided, the length of hospital stay less than 24 hours, imminent and inevitable death or the patient has been

Table 1 Clinical and laboratory signs of neonatal sepsis

Clinical signs of sepsis	► Abnormal body temperature (core temperature >38.5°C or <36°C and/or temperature instability); ► Cardiovascular instability (bradycardia (mean heart rate <10th percentile for age in the absence of external vagal stimulus, beta blockers or congenital heart disease or otherwise unexplained persistent depression over a 0.5–4 hour time period) or tachycardia (mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic unexplained persistent elevation over a 0.5–4 hour time period) and/or rhythm instability, reduced urinary output (<1 mL/kg/hour), hypotension (mean arterial pressure <5th percentile for age), mottled skin, impaired peripheral perfusion); ► Respiratory instability (apnoea episodes or tachypnoea episodes (mean respiratory rate >2 SD above normal for age) or increased oxygen requirements or requirement for ventilator support); ► Gastrointestinal (feeding intolerance, poor sucking, abdominal distension); ► Skin and subcutaneous lesions (petechial rash, sclerema); ► Non-specific (irritability, lethargy, hypotonia).
Laboratory signs of sepsis	► White cells <4×10^9 cells/L or >20×10^9 cells/L ► Immature to total neutrophil ratio (I/T) >0.2 ► Platelet count <100×10^9/L ► C reactive protein>15 mg/L or procalcitonin ≥2 ng/mL ► Glucose intolerance (hyperglycaemia (blood glucose >180 mg/dL or 10 mmol/L) or hypoglycaemia (blood glucose <45 mg/dL or 2.5 mmol/L)) ► Metabolic acidosis (base excess <–10 mEq/L or serum lactate >2 mmol/L)

previously recruited in the study. Investigators will review all of the mortality records during the time period of the study to try and identify how many of these cases may have been missed and whether there were any common characteristics in these participants.

Identification of participants

All doctors and nurses in the Department of Neonatology and Neonatal Intensive Care Unit of the study hospital will be informed about, and trained for, this clinical investigation. In addition, those working in the Department of Neonatology and Neonatal Intensive Care Unit will also be involved in the study. These staff will be trained to identify eligible patients and how to notify investigators.

Informed consent

Trained, Good Clinical Practice (GCP) accredited, members of the study team will collect informed consent. The team will discuss the study with the accompanying parent/guardian, or, if both parents are deceased or not actively involved in child care, the main long-term carer of the child will be accepted as the guardian and considered able to give consent for the study. Study staff will describe the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants and alternatives to enrolment. The parent/guardian will be invited to ask questions, which will be addressed by study staff, and they will be provided with appropriate contact numbers if they have any subsequent questions. If the parent/guardian agrees for the child to participate, they will be asked to sign and date an informed consent form. A copy of the patient information sheet and the informed consent form will be given to them to keep. In addition to the procedures above, illiterate signatories will have the informed consent form read to them in the presence of a witness who will sign to confirm this. The parent/guardian can withdraw from the study at any time (verbally) without affecting the care that the child will receive. If the parent/guardian decides at any time to take the child out of the study, no new information will be collected. However, information collected on the child up until that point will still be used. All patient information sheets and consent forms will be written in the local language and will use terms that are easily understandable.

Study procedures

An investigator will routinely record and collect demographic, clinical and laboratory information of the patients, the date of blood draw, the number of blood culture bottles inoculated, the result of the culture (whether positive or negative) and the susceptibility of the isolate to commonly used antimicrobials. Data from these records will be subsequently entered into CliRes Data Management System of OUCRU. These will be source data for this study. The number of patients admitted to the hospital annually will be obtained from hospital records. As part of this study, we request that all isolates from blood are stored and archived at –80°C. These isolates will be recultured and the identification will be reconfirmed. Selected isolated organisms from blood will have further molecular characterisation at a later date.

DATA COLLECTION

Demographic and clinical assessments

Data on neonatal sepsis at Children's Hospital 1 will be collected to the case report form. These data will include administrative data, demographic data, clinical characteristics, laboratory results, diagnoses, treatments and outcomes.

The Neonatal Therapeutic Intervention Scoring System will be used to estimate the disease severity.[23]

Microbiological assessments

Available routine microbiology data on neonatal bloodstream infections Children's Hospital 1. These data will include pathogenic agents isolated from blood culture and antimicrobial susceptibility profile of isolated bacteria (routine panel of antimicrobials). Selected isolates will have additional antimicrobial susceptibility testing, molecular analysis for antimicrobial resistance genes and genome sequencing of selected strains defined by antimicrobial susceptibility data.

LABORATORY METHODS

Microbiology testing

When required for checking or to non-routine antimicrobials, antimicrobial susceptibility testing of the pathogens isolated will be performed by disk diffusion using guidelines established by the Clinical and Laboratory Standards Institute and, when required, by minimum inhibitory concentration estimation using the VITEK 2 COMPACT automated machine. Antimicrobial susceptibilities tested will include nalidixic acid, ciprofloxacin, ceftriaxone, cefepime, ampicillin, trimethoprim–sulfamethoxazole, azithromycin, imipenem, colistin and amikacin for all Gram-negative organisms and oxacillin and vancomycin in Gram-positive organisms. The production of extended-spectrum beta lactamases (ESBL) will be investigated using the double-disc synergy test by comparing zone sizes between ceftazidime discs against ceftazidime–clavulanic acid discs and cefotaxime discs against cefotaxime–clavulanic acid discs. Isolates with an increase in diameter of inhibitory zone of equal to or more than 5 mm by the synergy of clavulanate will be considered ESBL positive.

Organisms including coryneforms (*Corynebacterium*, etc), *Micrococci*, *Propionibacterium*, *Bacillus*, alpha haemolytic *Streptococci*, environmental Gram-negative bacilli and non-pathogenic *Neisseria* will be considered potential contaminants. The pathogen-contaminant decision will be made based on the clinical relevance of the isolated bacteria and the independent assessments by two qualified medical microbiologists. If there is disagreement, then the case will be discussed until a decision is reached.

Bacterial storage

Organisms will be subcultured onto 5% blood agar and the purity of the isolate will be tested before storage in 20% glycerol at –80°C.

Isolation of nucleic acids

Isolates will be recultured and their identification rechecked. DNA will be extracted from bacterial isolates using the Wizard Genomic DNA Extraction Kit (Promega, Fitchburg, Wisconsin, USA). The quality and concentration of the DNA will be assessed using a nanodrop spectrophotometer prior to PCR amplification and the Quant-IT Kit (Invitrogen, Carlsbad, California, USA) prior to DNA sequencing.

PCR for resistance genes

The primary focus study is to investigate the distribution of antimicrobial resistance genes in bacteria causing neonatal sepsis. Therefore, all Gram-negative organisms will be investigated by PCR to detect genes catalysing resistance to cephalosporins, fluoroquinolones and carbapenems. Conventional PCR will be performed for the following classes of resistance genes using previously described methods. The multiplex and monoplex PCRs are described in these publications. PCR will be used to detect AmpC, ESBL (including CTX-M15), NDM, qnr, OXA, KPC and mecA/Van.[11 24–29]

Genome sequencing

Selected organisms (on the basis of their susceptibility profiles and resistance gene content) will be genome sequenced. We aim to sequence the greatest cross-section of organism groups as possible (ie, all *Staphylococci* or all *Klebsiella*). Selected bacterial isolates will be sequenced at OUCRU in Vietnam. Briefly, index-tagged paired end Illumina sequencing libraries will be prepared using one of 96 unique indexing tags as previously described. These will be combined into pools of uniquely tagged libraries and sequenced on the Illumina Genome Analyzer or HiSeq sequencer according to manufacturer's protocols to generate tagged 54–100 bp paired-end reads. This is previously approached for describing Gram-negative organisms and *Staphylococcus*.[20 30 31]

ANALYSIS PLAN

Statistical comparisons

Data will be presented in the form of tables and bar charts for descriptive variables, that is, number of organisms per year and number of AMR organisms per year. Statistical comparisons of features between groups (positive/negative blood culture, Gram-negative/Gram-positive bacteria, survival/non-survival, etc) and time trend analysis of the cultured isolates by month and the antimicrobial susceptibility patterns will be conducted. These data will be placed in the context of the broader population by comparison of these data with historical laboratory records of pathogens isolated from patients with bloodstream infections. Historical data from both neonates and older children will be analysed descriptively, and where appropriate, time trend analyses will be performed to determine significant alterations in bloodstream infection aetiology. All statistical analysis will be performed using Stata V.14 and R. P values of ≤0.05 will be considered significant.

Antimicrobial resistance genes and genome sequencing

The presence/absence of antimicrobial resistance genes will be reported as proportions per organism and then

stratified by organism, year and hospital department. Genome sequences will be determined to study phylogenetic relationships, the presence/absence of virulence genes and also AMR gene content and first analysed by species and then group by their Gram-stain results. Briefly, for phylogenetic analysis, chromosomal single nucleotide polymorphism (SNP) alleles will be concatenated for each strain to generate a multiple alignment of all SNPs. For maximum likelihood (ML) analysis, RAxML will be run using the generalised time-reversible model and 1000 bootstrap pseudoreplicate analyses were performed to assess support for the ML phylogeny. Root-to-tip branches will be extracted from the ML tree using the programme TreeStat. The relationship between root-to-tip distances and year of isolation will be analysed using linear regression. For Bayesian Evolutionary Analysis Sampling Trees (BEAST) analysis (V1.6), a GTR+Γ substitution model and defined tip dates as the date of isolation will be used.[30 31] To detect the presence or absence of genes read sets will be assembled using the de novo short read assembler Velvet and Velvet Optimizer. Organism specific read sets will then be aligned to the pangenome. Taxonomic investigation of accessory and AMR genes will be performed using MG-RAST V.3.2.

Ethics, regulatory approvals and governance

This study is sponsored by the University of Oxford and will be monitored by the Clinical Trials Unit at OUCRU. The Principal investigator (SB) will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki and the terms of approval of the appropriate ethical committees.[32] The study will be conducted in full conformity with relevant regulations and with the International Council on Harmonisation Guidelines for GCP.[33] This protocol and the relevant supporting document have already had the approvals of the Oxford Tropical Research Ethics Committee and the institutional review board of Children's Hospital 1. The investigators will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Dissemination and public engagement

Data from this study will be of interest to the scientific and clinical research communities. An informative resource for managing sepsis will be made available to local clinicians, clinical microbiologists and infection control policy developers. Study data will be reported according to the Strengthening the Reporting of Observational studies in Epidemiology guidance for reporting observational studies.[34] The authors (and their respective positions in the author list) will be agreed prior to the start of the study in accordance with the guidelines of the International Committee of Medical Journal Editors. In line with Wellcome Trust policy that the results of publicly funded research should be freely available, manuscripts arising from this study will be submitted to peer-reviewed journals which enable Open Access. In line with research transparency and greater access to data sharing policy of OUCRU in Vietnam will be implemented. This policy is based on a controlled access approach with a restriction on data release that would compromise an ongoing trial or study. Data exchange complies with Information Governance and Data Security Policies in all of the relevant countries.

DISCUSSION

As a conduit for introducing molecular biology for bacteriology into routine hospital care in Vietnam, the

Table 2 Study summary

Title	**The clinical features, antimicrobial susceptibility patterns and genomics of bacteria causing neonatal sepsis in a children's hospital in Vietnam**
Design	Observational prospective study All organisms isolated from blood will be stored and archived for molecular characterisation
Participants	Neonates (≤1 month of age) with sepsis
Planned enrolment period	2017–2019
Primary objectives	► To investigate the clinical characteristics of neonatal sepsis; ► To define the aetiology, the percentage of positive blood culture and major causes of sepsis; ► To investigate the antimicrobial susceptibilities of the pathogens causing sepsis and the rate of antimicrobial resistance; ► To measure the impact of sepsis on the severity of disease and the outcomes (mortality rate, length of stay and cost of treatment) of hospitalised neonates; ► To analyse the genome sequences of bacterial strains causing neonatal sepsis.
Secondary objectives	► To analyse the antimicrobial resistance profiles and gene distribution by clinical departments to add insight into the circulation of bacteria causing nosocomial infections; ► To study the genes catalysing resistance to the antimicrobials commonly used to treat neonatal sepsis (specifically third-generation/fourth-generation cephalosporins, fluoroquinolones and carbapenems).

study is unique and is planned to lead to future studies investigating appropriate empirical treatment for bacteraemia and the impact of AMR on the outcome of sepsis in the neonatal and paediatric population in Vietnam. By studying and defining disease aetiology, antimicrobial susceptibility patterns and disease outcome, we plan to develop an improved approach to managing bloodstream infections in our setting, and we will use these data to initiate intervention studies focused on preventing sepsis with AMR pathogens in neonates. Table 2 shows the summary of this study.

Duration and current status of study

The first patient was recruited in January 2017. At the current time, the recruitment is ongoing. The expected end date for recruitment is 31 December 2019. We expect to have completed our data analysis plan with a view of results by June 2020.

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Contributors All authors contributed to the conception and design of the study. NDT, TCD and SB drafted the protocol of the study. All authors read and critically revised the protocol and this article prior to submission.

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Competing interests None declared.

Patient consent Parental/guardian consent obtained.

Ethics approval Oxford (Oxford Tropical Research Ethics Committee 35-16) and Vietnam ().

Provenance and peer review Not commissioned; externally peer reviewed.

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