

## Burden of antibiotic resistance in infections among very-low-birthweight infants in Singapore

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### ABSTRACT

**Introduction:** Recent reports have described the increasing predominance of Gram-negative organisms among invasive bacterial infections affecting preterm infants. This changing pattern of infections is concerning due to the spread of antibiotic resistance among Gram-negatives.

**Method:** We conducted a single-centre, retrospective cohort study involving very-low-birthweight (VLBW) (<1500 grams) infants born <32 weeks gestation, with culture-proven infections (blood, urine, cerebrospinal fluid [CSF]) in the neonatal intensive care unit from 1 January 2005 to 31 October 2017.

**Results:** A total of 278 out of 2431 (11.4%) VLBW infants born <32 weeks gestation developed 334 infections, i.e. 52 (15.6%) early-onset infections (EOIs) and 282 (84.4%) late-onset infections (LOIs). The overall incidence decreased from 247 to 68 infections per 1000 infants over the study period, corresponding to reductions in LOI (211 to 62 infections per 1000 infants). A total of 378 bacteria were isolated, i.e. Gram-negatives accounted for 70.9% (45 of 59 [76.3%] EOI; 223 of 319 [69.9%] LOI). Specific resistant organisms were noted, i.e. Methicillin-resistant *Staphylococcus aureus* (8 of 21 *S. aureus* infections [38.1%]); Cephalosporin-resistant *Klebsiella* (18 of 62 isolates [29.0%]) and multidrug-resistant [MDR] *Acinetobacter* (10 of 27 isolates [37.0%]). MDR organisms accounted for 85 of 195 (43.6%) Gram-negative infections from the bloodstream and CSF. Based on laboratory susceptibility testing, only 63.5% and 49.3% of infecting bacteria isolated in blood were susceptible to empiric antibiotic regimens used for suspected EOI and LOI, respectively.

**Conclusion:** Gram-negative bacteria are the predominant causative organisms for EOI and LOI and are frequently MDR. Understanding the pattern of antimicrobial resistance is important in providing appropriate empiric coverage for neonatal infections.

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**Keywords:** bacteremia, Gram-negative bacilli, microbial drug resistance, nosocomial infection, premature infant

### CLINICAL IMPACT

#### What is New

- In our setting with a high incidence of Gram-negative early- and late-onset infections among preterm very-low-birthweight infants, multidrug-resistant organisms were relatively common (47% of isolates).
- Between one third and half of all bacteria isolates in the blood were resistant to the empiric antibiotic regimen used for suspected early- and late-onset infections.

#### Clinical Implication

- There is a need for continued monitoring of antimicrobial resistance among infecting organisms in this high-risk population to inform infection control initiatives.

### INTRODUCTION

Recent reports have described an increased predominance of Gram-negative organisms (*Escherichia coli*, *Klebsiella* species [spp]) among invasive bacterial infections in early- and late-onset neonatal sepsis in many settings.<sup>1-5</sup> This changing pattern of infections is particularly concerning due to the global spread of drug resistance among Gram-negatives, leading to increasingly limited therapeutic options.<sup>6,7</sup> The ongoing worldwide spread of antimicrobial resistance represents a major challenge in neonatal care. Previous studies have estimated that multidrug-resistant (MDR) bacteria account for approximately 30% of global neonatal sepsis mortality.<sup>8</sup>

Nearly half of pathogens causing neonatal sepsis were reported to be resistant to first-line (ampicillin or penicillin, gentamicin) and second-line (third-generation cephalosporin) World Health

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Organization-recommended treatments.<sup>9,10</sup> Of note, preterm infants admitted to the neonatal intensive care unit (NICU) are at particularly high risk of colonisation and infection with these MDR microorganisms.<sup>11–13</sup> There is a current dearth of local and regional data on the prevalence of antibiotic resistance and its impact on empiric antibiotic coverage among preterm, very-low-birthweight (VLBW) infants. Selection of empiric antimicrobial therapy for suspected bacterial infection or sepsis in the NICU requires knowledge of the local epidemiology of infecting organisms and their associated antibiotic resistance patterns. As such, we aim to explore the epidemiology of infecting microorganisms and their associated antimicrobial resistance in the NICU, over a 13-year period.

## METHOD

### Study design, setting and participants

This is a retrospective cohort study of VLBW (<1500 g) infants born <32 completed weeks gestational age over a 13-year period (1 January 2005–31 October 2017), who were admitted to the NICU at KK Women's & Children's Hospital, Singapore. Infants with major congenital anomalies, stillbirths and labour-room deaths were excluded.

### Data sources

Our VLBW clinical database records maternal, perinatal and neonatal information using a standardised data collection form for all live-born infants <1500 g in the hospital.<sup>1</sup> We identified all VLBW infants who were born <32 weeks gestation with positive blood, urine and cerebrospinal fluid (CSF) culture results from our hospital microbiology database. We subsequently performed a data linkage with our VLBW database using unique national identification numbers (allocated to every baby born in Singapore). We included data on all infants until initial birth discharge.

### Variables and definitions

Early-onset infection (EOI) and late-onset infection (LOI) were defined as clinical episodes with  $\geq 1$  positive blood, urine and/or CSF culture in the presence of signs or symptoms suggestive of infection at <72 and  $\geq 72$  hours of life, respectively. Positive cultures with coagulase-negative staphylococci (CoNS), *Micrococcus*, *Bacillus*, *Corynebacterium* and *Propionibacterium* species were considered contaminants unless  $\geq 2$  cultures were positive for the organism and/or the infant showed signs of sepsis and received intravenous antibiotics for  $\geq 5$  days.

A separate infection episode was considered if the infant developed signs of sepsis with a

positive blood, urine or CSF culture after completing at least 10 days of appropriate antibiotics. Mortality was attributed to the infection if it was designated as the primary cause of death by the attending physician and occurred within 7 days from initiation of antibiotics.<sup>14</sup> The empiric antibiotic regimen used during the study period were penicillin and gentamicin (EOI), and cloxacillin and gentamicin (LOI), with escalation to third-generation cephalosporin or carbapenem if clinically indicated.

Gestational age is defined as the best obstetric estimate of completed weeks based on obstetric history, clinical examination and antenatal ultrasound. An infant is small-for-gestational age (SGA) if birth weight is <10th percentile according to the Fenton growth charts.<sup>15</sup> Prolonged rupture of membranes (PROM) is defined as the rupture of membranes >18 hours prior to birth. Histologic chorioamnionitis is defined as the presence of inflammatory cells in the chorioamniotic membrane, umbilical cord and/or the placental disc.<sup>16</sup> Severe morbidities included were necrotising enterocolitis  $\geq$  stage 2,<sup>17</sup> severe intraventricular haemorrhage (grades 3–4),<sup>18</sup> bronchopulmonary dysplasia<sup>19</sup> and severe retinopathy of prematurity (stages 3–5).<sup>20</sup>

Identification of all causative bacteria was performed using standard microbiologic methods, and antibiotic susceptibility was determined according to the Australian calibrated dichotomous sensitivity (CDS) antimicrobial susceptibility testing standards.<sup>21</sup> MDR Gram-negative bacteria were defined as those resistant to at least 1 agent belonging to at least 3 of the following antibiotic categories: carbapenems (imipenem, meropenem), penicillins (ampicillin, piperacillin-tazobactam), broad-spectrum cephalosporins (ceftazidime, cefepime), aminoglycosides (gentamicin, amikacin), and fluoroquinolones (ciprofloxacin).<sup>22,23</sup> Specific MDR bacteria of interest included (1) Methicillin-resistant *Staphylococcus aureus* (MRSA): *S. aureus* resistant to oxacillin, cefoxitin or methicillin; (2) Vancomycin-resistant *Enterococcus* (VRE): *Enterococcus* spp that is resistant to vancomycin; (3) Cephalosporin-resistant *Klebsiella* spp (CephR-*Klebsiella*): *Klebsiella* spp testing non-susceptible to ceftazidime, cefotaxime, ceftriaxone or cefepime; (4) Carbapenem-resistant *Enterobacteriaceae*: any *E. coli*, *Klebsiella* spp or *Enterobacter* spp testing resistant to carbapenems; (5) MDR *Acinetobacter*: any *Acinetobacter* spp testing non-susceptible to at least 1 agent in at least 3 out of 6 antimicrobial classes.<sup>23,24</sup> When a bacteria species has known intrinsic resistance to an antibiotic category, that specific antibiotic category was not considered in calculating the number of categories to which that bacteria species is non-susceptible.<sup>23</sup>

## Statistical analysis

Differences in proportions between categories were tested using chi-squared test or Fisher's Exact test (where appropriate) and Mann-Whitney U test for categorical and continuous variables, respectively. Univariable and multivariable logistic regression analyses were performed to determine associated factors for the outcomes of death and death and/or severe morbidity. The following predetermined variables were included in the multivariable models: sex, birthweight, receipt of antenatal steroids, and MDR organisms. Quantitative associations from logistic regression were reported as adjusted odds ratios (AORs) with 95% confidence intervals (CIs). All tests were two-sided, and *P* value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 23.0 (IBM Corp, NY, US).

This study protocol was reviewed and approved by the SingHealth Centralised Institutional Review Board.

## RESULTS

Over the 13-year period, 278 out of 2431 (11.4%) VLBW infants born <32 weeks gestation developed

334 episodes of culture-confirmed infections. Of these, 52 (15.6%) episodes were EOIs and 282 (84.4%) were LOIs. Five infants had both EOIs and LOIs during their admission. Another 56 infants had multiple infection episodes prior to discharge: 41 with 2 episodes, 9 with 3 episodes, and 6 with ≥4 episodes. The clinical characteristics of infants with EOIs and LOIs are shown in Table 1. Compared to infants who developed LOIs, infants with EOIs had significantly higher rates of PROM, histologic chorioamnionitis and birthweight but lower proportion of SGA. The overall incidence of infections decreased from a peak of 247 to 68 infections per 1000 infants over the study period (Fig. 1). The overall decline was mirrored by the decrease in LOI, from a peak of 211 to 62 infections per 1000 infants. The EOI incidence remained largely unchanged, ranging from 5 to 37 infections per 1000 infants.

Of the 334 infections diagnosed, 275 (82.3%) were blood culture positive and 59 (17.7%) urine culture positive. All EOIs were blood culture positive infections. All 8 CSF-positive cultures were associated with blood culture positive infections, and 7 out of 8 (88%) were LOIs. Excluding 5 infants who had both episodes of EOIs and LOIs,

Table 1. Clinical characteristics of infants with early- and late-onset infections.

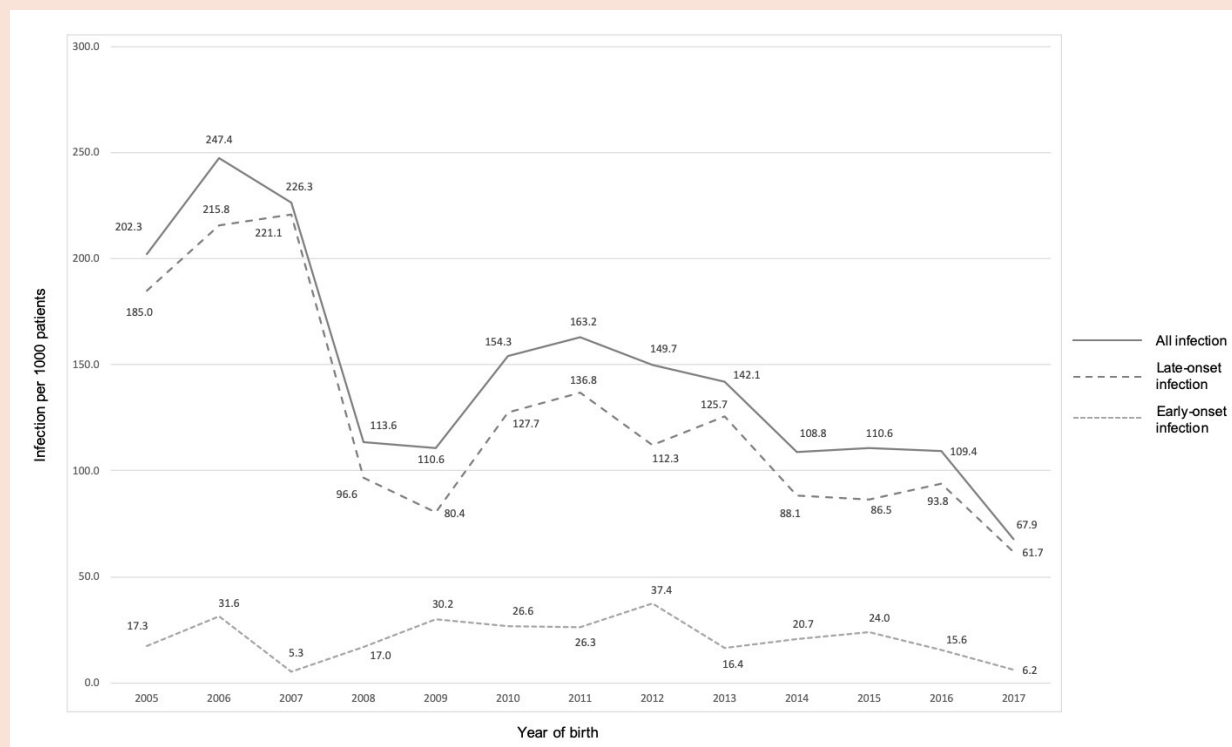
|                                         | EOI<br>(n=52)        | LOI *<br>(n=231) |
|-----------------------------------------|----------------------|------------------|
| Median maternal age, years (IQR)        | 32 (29–35)           | 32 (27–35)       |
| Abruptio placentae, no. (%)             | 8 (15.4)             | 40 (17.3)        |
| Pregnancy-induced hypertension, no. (%) | 5 (9.6)              | 45 (19.5)        |
| PROM, no. (%)                           | 28 (53.8)            | 64 (27.7) **     |
| Antenatal steroids, no. (%)             | 45 (86.5)            | 206 (89.2)       |
| Histologic chorioamnionitis, no. (%)    | 40 (76.9)            | 93 (40.8) **     |
| Male sex, no. (%)                       | 25 (48.1)            | 137 (59.3)       |
| Vaginal delivery, no. (%)               | 27 (51.9)            | 112 (48.5)       |
| Outborn, no. (%)                        | 1 (1.9)              | 15 (6.5)         |
| Median birthweight, grams (IQR)         | 972.5 (721.5–1184.5) | 780 (650–940) ** |
| SGA, no. (%)                            | 4 (7.8)              | 55 (23.8) **     |
| Gestational age <28 weeks, no. (%)      | 36 (69.2)            | 174 (75.3)       |
| Median gestational age, weeks (IQR)     | 27 (25–28)           | 26 (24–27)       |
| Multiple gestation, no. (%)             | 9 (17.3)             | 56 (24.2)        |
| 5-minute Apgar <7, no. (%)              | 17 (33.3)            | 50 (21.8)        |

\* 5 infants had EOI and LOI during the course of their admission

\*\* *P*<0.05 for comparisons between EOI and LOI

EOI: early-onset infection; IQR: interquartile range; LOI: late-onset infection; PROM: prolonged rupture of membrane; SGA: small-for-gestational age

Fig. 1. Trends of infections (early and late onset) in the NICU over the study period.



the rate of all-cause mortality was 36.2% for those with EOIs compared to 21.7% for those with LOIs (Supplementary Table S1). This is in contrast to the higher incidence of severe morbidity among infants who had LOIs (70.8%) compared to EOIs (37.0%).

A total of 378 organisms were isolated from 334 infections (Table 2). There was a predominance of Gram-negative infections, accounting for 70.9% of all bacteria isolated, including 45 out of 59 (76.3%) of all EOIs and 223 out of 319 (69.9%) of LOIs. *E. coli* (65 of 378, 24.2%), *Klebsiella* spp (62 of 378, 23.1%), *Enterobacter* spp (53 of 378, 19.8%), *Acinetobacter* spp (29 of 378, 10.8%) and *Pseudomonas* spp (22 of 378, 8.2%) accounted for more than 60% of all Gram-negative isolates. The dominance of Gram-negative EOI and LOI over the 13-year period was illustrated by the general trends of higher annual proportion of this subtype, ranging from 40% to 100% of EOI and from 48% to 89% for LOI (Supplementary Fig. S1).

A total of 239 infections with *S. aureus*, *E. fecalis*, *Klebsiella* spp, *E. coli*, *Enterobacter* spp and *Acinetobacter* spp were analysed for specific resistance patterns (Supplementary Table S2). The following incidences were noted: MRSA (8 of 21 *S. aureus* infections [38.1%]); cephalosporin-resistant *Klebsiella* (18 of 62 isolates [29.0%]) and MDR-*Acinetobacter* (10 of 27 isolates [37.0%]). There were no carbapenemase-resistant *Enterobacteriaceae* (*E. coli*, *Klebsiella*, *Enterobacter*; total of

172 isolates tested) or VRE (total of 16 isolates tested). MDR organisms accounted for 85 of 195 (43.6%) Gram-negative infections isolated from the blood stream and CSF (Table 3). Of these, *Enterobacter* spp and *Acinetobacter* spp infections had high rates of MDR, i.e. 91.2% and 37.0%, respectively.

Up to 63.5% and 49.3% of infecting bacteria isolated in the blood were susceptible to the empiric antibiotic regimen used for suspected EOI (benzylpenicillin and gentamicin) and LOI (cloxacillin and gentamicin), respectively (Table 4). If cloxacillin and amikacin were used for LOI, up to 70.4% of the bacteria isolated would be expected to be susceptible. There was no statistically significant difference in the proportion of resistant infecting organisms to empiric coverage between those who died (9 of 19, 47%) and those who survived EOI (9 of 33, 27.3%;  $P=0.1$ ). Similarly, there was no difference in the proportion of resistance to empirical coverage between those who died (27 of 57, 47.4%) and survived LOI (89 of 174, 51.1%;  $P=0.6$ ). Additionally, after adjusting for confounders, episodes of infections with MDR Gram-negative organisms were not significantly associated with increased odds of death (AOR 1.0; 95% CI 0.4, 2.4) or the combined outcome of death and/or severe morbidity (AOR 0.5; 95% CI 0.2, 1.7) (Supplementary Table S3).

Table 2. Microbial distribution of early- and late-onset infections.

| Microorganism                           | EOI (n=59) | LOI (n=319) | Total (n=378) |
|-----------------------------------------|------------|-------------|---------------|
| Gram positive, no. (%)                  | 14 (23.7)  | 96 (30.0)   | 110 (29.1)    |
| CoNS                                    | 4 (28.6)   | 48 (50.0)   | 52 (47.3)     |
| <i>Staphylococcus aureus</i>            | 1 (7.1)    | 20 (20.8)   | 21 (19.1)     |
| <i>Enterococcus faecalis</i>            | 1 (7.1)    | 15 (15.6)   | 16 (14.5)     |
| GBS                                     | 6 (42.9)   | 6 (6.3)     | 12 (10.9)     |
| Others                                  | 2 (14.3)   | 7 (7.3)     | 9 (8.3)       |
| Gram negative, no. (%)                  | 45 (76.3)  | 223 (70.0)  | 268 (70.9)    |
| <i>Escherichia coli</i>                 | 23 (51.1)  | 42 (18.8)   | 65 (24.2)     |
| <i>Klebsiella</i> spp                   | 5 (11.1)   | 57 (25.6)   | 62 (23.1)     |
| <i>Enterobacter</i> spp                 | 5 (11.1)   | 48 (21.5)   | 53 (19.8)     |
| <i>Acinetobacter</i> spp                | 2 (4.4)    | 27 (12.1)   | 29 (10.8)     |
| <i>Pseudomonas</i> spp                  | 5 (11.1)   | 17 (7.6)    | 22 (8.2)      |
| <i>Serratia marcescens</i>              | 0          | 16 (7.2)    | 16 (6.0)      |
| <i>Elizabethkingia meningoseptica</i>   | 0          | 6 (2.7)     | 6 (2.2)       |
| <i>Stenotrophomonas maltophilia</i>     | 0          | 3 (1.3)     | 3 (1.1)       |
| <i>Chryseobacterium meningosepticum</i> | 0          | 3 (1.3)     | 3 (1.1)       |
| <i>Citrobacter koseri</i>               | 1 (2.2)    | 1 (0.5)     | 2 (0.7)       |
| <i>Burkholderia cepacia</i>             | 0          | 1 (0.5)     | 1 (0.4)       |
| <i>Flavobacterium odoratum</i>          | 1 (2.2)    | 0           | 1 (0.4)       |
| <i>Haemophilus influenzae</i>           | 1 (2.2)    | 0           | 1 (0.4)       |
| <i>Moraxella</i> spp                    | 0          | 1 (0.5)     | 1 (0.4)       |
| <i>Morganella morganii</i>              | 1 (2.2)    | 0           | 1 (0.4)       |
| <i>Pantoea agglomerans</i>              | 0          | 1 (0.5)     | 1 (0.4)       |
| <i>Proteus mirabilis</i>                | 1 (2.2)    | 0           | 1 (0.4)       |

CoNS: coagulase-negative *Staphylococcus*; EOI: early-onset infection; GBS: Group B *Streptococcus*; LOI: late-onset infection; spp: species

Table 3. Distribution of multidrug-resistant organism among Gram-negative organisms isolated from blood and cerebrospinal fluid samples.

| Microbe                                | No. of isolates | MDR, no. (% of isolate) |
|----------------------------------------|-----------------|-------------------------|
| <i>Escherichia coli</i>                | 49              | 12 (24.5)               |
| <i>Klebsiella</i> spp                  | 48              | 16 (33.3)               |
| <i>Enterobacter</i> spp                | 34              | 31 (91.2)               |
| <i>Acinetobacter</i> spp               | 27              | 17 (63.0)               |
| <i>Pseudomonas</i> spp                 | 16              | 0                       |
| <i>Serratia marcescens</i>             | 8               | 5 (62.5)                |
| <i>Elizabethkingia meningoseptica</i>  | 3               | 3 (100)                 |
| <i>Chryseobacterium meningoseptica</i> | 3               | 3 (100)                 |
| <i>Stenotrophomonas maltophilia</i>    | 2               | 2 (100)                 |
| <i>Proteus mirabilis</i>               | 1               | 0                       |
| <i>Morganella morganii</i>             | 1               | 1 (100)                 |
| <i>Flavobacterium odoratum</i>         | 1               | 1 (100)                 |
| <i>Citrobacter koseri</i>              | 1               | 0                       |
| <i>Burkholderia cepacia</i>            | 1               | 1 (100)                 |
| Total                                  | 195             | 92 (47.2%)              |

MDR: multidrug resistant

Table 4. Susceptibility of bloodstream bacteria isolated during infection episodes to empiric antimicrobial combinations over the study period.

| Antibiotic regimens                                | 2005            | 2006            | 2007            | 2008           | 2009           | 2010            | 2011            | 2012            | 2013            | 2014            | 2015            | 2016            | 2017          | Total             |
|----------------------------------------------------|-----------------|-----------------|-----------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------|-------------------|
| <b>Early-onset infection</b>                       |                 |                 |                 |                |                |                 |                 |                 |                 |                 |                 |                 |               |                   |
| Benzylpenicillin + gentamicin, no. (% susceptible) | 1 of 3 (33.3)   | 4 of 6 (66.7)   | 0 of 1 (0)      | 1 of 3 (33.3)  | 4 of 6 (66.7)  | 4 of 5 (80.0)   | 3 of 5 (60.0)   | 6 of 7 (85.7)   | 1 of 3 (33.3)   | 4 of 4 (100)    | 2 of 5 (40.0)   | 2 of 3 (66.7)   | 1 of 1 (100)  | 33 of 52 (63.5)   |
| <b>Late-onset infection</b>                        |                 |                 |                 |                |                |                 |                 |                 |                 |                 |                 |                 |               |                   |
| Cloxacillin + gentamicin, no. (% susceptible)      | 11 of 23 (47.8) | 9 of 25 (36.0)  | 15 of 25 (60.0) | 7 of 13 (53.8) | 5 of 12 (41.7) | 5 of 17 (29.4)  | 5 of 17 (29.4)  | 10 of 19 (52.6) | 9 of 20 (45.0)  | 9 of 12 (75.0)  | 8 of 16 (50.0)  | 12 of 15 (80.0) | 5 of 9 (55.6) | 110 of 223 (49.3) |
| Cloxacillin + amikacin, no. (% susceptible)        | 14 of 23 (60.9) | 14 of 25 (56.0) | 21 of 25 (84.0) | 7 of 13 (53.8) | 5 of 12 (41.7) | 10 of 17 (58.8) | 14 of 17 (82.4) | 17 of 19 (89.5) | 13 of 20 (65.0) | 10 of 12 (83.3) | 13 of 16 (81.3) | 12 of 15 (80.0) | 7 of 9 (77.8) | 157 of 223 (70.4) |

Episodes with multiple organisms are considered susceptible only if all organisms are susceptible.

## DISCUSSION

Recent reports from several large cohort studies located in the US, Australia and New Zealand have revealed similar trends of stable EOI and decreasing LOI incidence among preterm, VLBW infants in the NICU.<sup>25–28</sup> The reported rates of neonatal sepsis ranged from 1.0% to 1.9% for EOI and from 12.2% to 24.5% for LOI,<sup>27,28</sup> which are consistent with our contemporaneous cohort. These reports have highlighted the increasing trend of Gram-negative EOI among VLBW preterm cohorts, with a predominance of *E. coli* over Group B *Streptococcus*.<sup>27,28</sup> The higher incidence of Gram-positive LOI, specifically CoNS, in these studies<sup>25,28,29</sup> is in contrast to our study and several reports from this region.<sup>4,30–32</sup> We observe a higher rate of Gram-negative LOI, with the most common organisms being *E. coli* and *Klebsiella pneumoniae*. Our study also illustrates a consistent trend of Gram-negative dominance for EOI and LOI in our unit through the past 13 years.

Several reasons have been proposed for this difference in microbial epidemiology, including the high antibiotic usage during the antepartum and perinatal periods.<sup>2,7</sup> Early life exposure can lead to alterations in neonatal mucosal colonisation, which may lead to an increased risk of Gram-negative infections.<sup>13,33</sup> The predominance of Gram-negative infections in this high-risk population is concerning due to the emergence of MDR Gram-negative bacteria globally, with the associated increased risk for mortality.<sup>8,34</sup> Preterm infants in the NICU are at particular risk for MDR infection due to high rates of MDR colonisation,<sup>11</sup> prolonged hospitalisation, high usage of extended spectrum antibiotics,<sup>35</sup> poor immune function and high usage of invasive devices.<sup>31</sup> Available studies from Asia, South America and Africa have reported that between 50% and 80% of infants in these settings are colonised with MDR Gram-negative bacteria with high reported rates of resistance to antibiotic such as ampicillin, aminoglycoside and third-generation cephalosporins.<sup>31</sup> The proportion of MDR Gram-negative organisms in our study is moderately high (47.2% of infecting organisms) compared to similarly reported MDR rates from around the region, i.e. Taiwan (18.6%)<sup>22</sup> and India (80–100%).<sup>36,37</sup>

The incidence of antibiotic resistance to empiric antibiotic regimens is important to determine in the clinical setting, as inappropriate coverage may contribute to worse outcomes. It is particularly concerning to note that our empiric regimen of benzylpenicillin and gentamicin only covered up to 64% of infecting EOI organisms in our study over the study period. Coverage with cloxacillin and gentamicin for infecting LOI organisms was lower, with only 49.3% coverage. As expected, the

majority of nonsusceptibility to empiric coverage in these infections was related to Gram-negative organisms. Even so, the percentage of sepsis-related mortality (EOI 28.8%, LOI 7.5%) in our setting was within the range of reported incidence in the literature over the study period.<sup>27, 28</sup> Moreover, there was no difference in the proportion of resistance to empiric coverage among those infants who survived and died from EOI and/or LOI. This could be related to our unit practice of early escalation to a third-generation cephalosporin or meropenem among patients with clinical evidence of sepsis. Both of these reserve antibiotics are also still mostly effective against the common infecting Gram-negative organisms. Of note, our empiric antibiotic regimen for LOI has been changed to cloxacillin and amikacin in recent times to provide better coverage for these organisms while keeping broad spectrum antibiotic usage in check.

Reports on resistance to routinely used first-line antibiotics for neonatal infections vary across different geographical regions. A multicentre US NICU study of 6184 infants demonstrated that around 1 quarter of all infecting Gram-negative isolates were non-susceptible to  $\geq 1$  antibiotic, with up to 14.8% being resistant to gentamicin, 9.9% to third-generation cephalosporin or cefepime, and 0.5% (0–1.1%) to carbapenems.<sup>33</sup> Similar patterns of resistance were noted in a separate surveillance network study from the UK where the antibiotic resistance of *E. coli* and *Klebsiella* spp to the recommended antibiotic empiric regimen for EOI and LOI was 6–16% and 8–12%, respectively.<sup>38</sup> The rates of Gram-negative gentamicin resistance in our study were much higher (37% resistance) and are similar to that previously reported in 4 Asian NICUs.<sup>4</sup> Importantly, the rates of amikacin and cefotaxime resistance were lower in our Gram-negative isolates in comparison to that reported from regional neonatal units.<sup>4,39–42</sup> Carbapenem resistance is also uncommon in our setting with the only notable resistance among *Acinetobacter* spp infections. Among the Gram-positive microorganisms isolated, CoNS and *S. aureus* accounted for almost 75% of such infections. Our reported rates of MRSA infections were similar to that reported from surrounding neonatal units with rates ranging from 41% to 45%.<sup>40,41</sup> We did not detect any vancomycin-resistant isolates among the *Enterococcus* infections.

Our results are limited by the single-centre design of our study. However, our 13-year cohort is drawn from the largest NICU in Singapore that provides care for an estimated two thirds of VLBW infants born locally. This provides a reasonable basis from which to make important comparisons and

inferences about local and regional burden of infections and antimicrobial resistance. Lack of complete data on antenatal antibiotic exposure and device-associated infections precluded further analysis of its impact on risk of infection and resistance patterns. It is also important to note that this study is based on in vitro reporting of susceptibility to individual antimicrobial agents that does not account for the potential synergistic effect of antimicrobial combinations. Additionally, the susceptibility data were extracted from reports generated for clinical use, which may be influenced by reporting standards<sup>21</sup> that are used to define resistant organisms that may not be solely defined by in vitro testing.

These data provide important insights into the local and regional burden of infections among preterm VLBW infants and the NICU as well as an understanding of the general patterns of antimicrobial resistance of infecting organisms. These data can form the basis from which to develop infection control initiatives and adjustment of antimicrobial coverage in this high-risk population.

## CONCLUSION

In our setting, Gram-negative bacteria are the predominant causative organisms for EOI and LOI and are frequently MDR. Understanding the pattern of antimicrobial resistance is important in providing appropriate empiric coverage for neonatal infections.

## Ethics approval

*This study protocol was reviewed and approved by the local institutional review board. The study was performed in accordance with the SingHealth Centralised Institutional Review Board (Reference nos. 2015/2992 and 2022/2194). It was performed in accordance with the Declaration of Helsinki. The study was granted an exemption of written consent by the ethics review board.*

## Conflict of interest

*The authors declared no conflicts of interest.*

## Funding

*This study received no funding.*

## Data availability statement

*Data are available upon reasonable request from the corresponding author.*

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