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Delineating the risk factors, sources of acquisition and clinical outcomes in neonates colonised with multidrug-resistant bacteria: The NeoCol study

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Abstract

Antimicrobial resistance contributes to neonatal mortality globally. Delineating transmission pathways resulting in neonatal colonisation with multidrug-resistant (MDR) bacteria is necessary to reduce this burden. We conducted a prospective cohort study of 189 mother-infant dyads to identify the risk factors associated with transmission of MDR bacteria, revealing a high prevalence of maternal colonisation with MDR bacteria at delivery (63%, 120/189), and in neonates by hospital discharge (61%, 115/189). However, maternal colonisation with carbapenem-resistant Enterobacterales (CRE) was low (3%, 6/189) whereas CRE acquisition occurred at a median time point of 13 days in hospitalised neonates (26%, 50/189), suggesting horizontal transmission of CRE predominates. Compared to non-colonised neonates, those colonised with MDR bacteria were more likely to have been exposed to antibiotics prior to colonisation (63%, vs 24%, $p<0.001$), require a longer hospital stay (16 days vs 2 days, $p<0.001$) and have episodes of sepsis following colonisation (62% vs 24%, $p<0.001$). However, most culture-positive neonatal infections (91%, 10/11) were not caused by previously identified colonising bacteria. Our study reveals poor clinical outcomes associated with MDR neonatal colonisation, and suggests improved antibiotic stewardship strategies may reduce this burden.

Introduction

Neonatal sepsis is a major contributor to child mortality worldwide, with the most significant burden evident in resource-constrained healthcare settings, where antimicrobial resistance (AMR) increasingly drives higher infant mortality rates.[1–3] Gram-negative bacteria now predominate as the pathogens causative of neonatal sepsis globally, and frequently exhibit antibiotic resistance mechanisms.[4,5] Consequently, neonatal sepsis is increasingly difficult to treat due to the rising prevalence of multidrug-resistant (MDR) infections, which are associated with poorer morbidity and mortality outcomes.[6,7] This is of particular concern in low- and middle-income countries (LMICs), where empirical antibiotic regimens recommended by many global guidelines are curated around data from high-income healthcare settings, and provide poor coverage against the MDR bacteria increasing causative of neonatal sepsis.[3,5,8–13]

Delineating the epidemiology of neonatal sepsis is challenging in many LMICs, due to the significant clinical load posed by the population density in many of these settings, particularly within Southeast Asia.[8] This high clinical load is compounded by challenges in inequitable laboratory capacity and the limited diagnostic yield of blood cultures in neonatal infections, particularly due to high rates of empirical perinatal antibiotic prescribing, and the difficult in obtaining adequate blood culture in this population - factors that impact the ability to diagnose neonatal infections across all healthcare settings.[14–16] While tools such as sepsis calculators have been developed to improve the diagnostic sensitivity and specificity of neonatal sepsis, their reliance on key informative data (such as maternal GBS colonisation status) limits their utility in settings where this information may not be routinely available, which also corresponds to settings with a high prevalence of neonatal sepsis and AMR.[17]

The transmission dynamics that predispose to colonisation and infection in neonates are poorly understood, particularly for gram-negative bacteria.[18] Whilst maternal colonisation is a well-recognised risk factor of neonatal Group B Streptococcal infection, the relative contribution of vertical versus horizontal transmission of gram-negative bacteria is far less clear.[19] Prior studies have suggested multidrug-resistant gram-negative bacterial (MDR-GN) colonisation may increase the risk of subsequent invasive infections,[19,20] and these principles inform resource-intensive infection, prevention and control (IPC) strategies around precaution-based care. To mitigate this risk, clinicians are also inclined to prescribe broad-spectrum antibiotic therapy when a neonate known to be colonised with MDR-GN bacteria becomes unwell, which may propagate the selection pressure of further MDR pathogens.[7,20]

However, the evidence implying bacterial colonisation may increase the risk of subsequent invasive infection with correlating pathogens is scarce; as is the clarity identifying the risk factors that predispose to neonatal

MDR-GN colonisation and the pathways by which this may occur.[21] Neonatal intensive care units (NICUs) have been identified as environments where MDR-GN pathogens may readily spread, causing late-onset infections in neonates requiring prolonged hospitalisation in both resource-rich and resource-constrained healthcare settings.[18,22,23] Recent studies evaluating colonisation dynamics in hospitalised neonates in Kenya, Nigeria, Thailand, and Cambodia have specifically demonstrated rapid acquisition of MDR-GN pathogens in hospitalised neonates,[22,24–26] influenced by the prevalence of maternal MDR-GN colonisation, mode of delivery, antibiotic prescribing practices, the number and colonisation status of other neonates in the NICU, and community factors such as household density and access to safe water and sanitation.[21,24,25,27] By contrast, a comprehensive study conducted in India found no evidence that maternal MDR-GN colonisation was the source of subsequent neonatal sepsis, and hospital-acquired pathogens were predominantly identified as causative of invasive neonatal infections.[18] Together, these studies suggest that unlike for GBS, hospital-acquired (horizontal) transmission of MDR-GN bacteria are a more important source of invasive infection in neonates, although there are scant data to support these transmission dynamics, and the impact colonisation may (or may not) have on subsequent invasive infection.[21,28]

Uncertainty surrounding transmission pathways around the increasing prevalence of MDR-GN bacteria as causative pathogens in neonatal sepsis impedes the development of effective interventions to reduce neonatal mortality globally. Improving our understanding of how, when and why MDR-GN pathogens colonise and infect neonates is crucial; especially in high-burden, LMIC settings. This study aimed to address these knowledge gaps, to identify the acquisition pathways of MDR-GN colonisation between vertical and environmental sources in an urban tertiary hospital in the highly populous country of Indonesia, where AMR poses a significant challenge.[5,12] Alongside identifying transmission pathways of MDR-GN bacteria, we sought to identify the risk factors associated with neonatal and maternal MDR-GN colonisations, and the impact this colonisation may have on subsequent invasive infections and mortality.

Results

A total of 189 mother–infant dyads were enrolled in the study (Supplementary Figure 1). The gestational age of included infants ranged from 24 to 41 weeks. Overall, 115 infants (of 189, 61%) and 120 mothers (of 189, 63%) were colonised during the study period with either extended-spectrum beta-lactamase (ESBL)-producing bacteria, carbapenem-resistant Enterobacterales (CRE) or a non-fermenting carbapenem-resistant gram-negative pathogen (CR-GN), such as *Acinetobacter baumannii* or *Pseudomonas aeruginosa* (Figure 1).

At delivery, 14 neonates (of 189, 8%) were colonised with an ESBL-producing GNB, rising to 51% (97/189) by hospital discharge. Four neonates were colonised with CRE at birth (of 189, 2%) increasing to 26% by discharge (50/189), and two (of 189, 1%) were colonised with another CR-GN at delivery, rising to 16% (30/189) by discharge. Postnatal colonisation occurred at a mean of 8.8 days of age, with ESBL-producing pathogens acquired more quickly (mean: 10.5 days) than CRE (13.1 days). (Figure 2).

Klebsiella pneumoniae (n=112), *Escherichia coli* (n=37), and *A. baumannii* complex (n=30) were the most frequently identified pathogens identified in neonates (Figure 3). Among mothers, the predominant pathogens identified were *E. coli* (=58), *K. pneumoniae* (n=37) and *A. baumannii* (n=19) (Figure 3, Supplementary Tables 1-3). Among 65 mother-infant dyads where both were colonised with a pathogen, phenotypically-matched ESBL-producing pathogens were evident in 55 dyads, whilst co-colonisation with CR-GN was evident in nine dyads (including six dyads both colonised with *A. baumannii*). Only one mother-infant dyad was co-colonised with CRE (Supplementary Table 4).

Lower segment caesarean section (LSCS) was the most common mode of delivery, occurring in 72% (136/189) of enrolments. Among infants enrolled at birth, 117 (of 189, 62%) were subsequently admitted to the NICU. The median duration of admission was five days (interquartile range [IQR] 2 to 23 days).

Risk factors for maternal colonisation with MDR-GN bacteria:

Of the 189 women included in the study, 120 (of 189, 63%) were colonised with an ESBL (120, 55%) and/or CRE (6, 3%) and/or CR-GN (33, 17%) (Table 1). There were no significant differences in maternal age ($p > 0.9$), education level ($p > 0.9$), employment status ($p = 0.5$), or household income ($p > 0.9$) in colonised vs non-colonised women. Similarly, household density, access to safe water, toilet and handwashing facilities, and bathing frequency did not differ between groups (Supplementary Table 5).

Risk Factors for Neonatal Colonisation

Compared with non-colonised neonates (n = 74), those colonised had a significantly lower median birthweight (2,035g [IQR 1,490–2,790g] vs 2,595 g [IQR 2,195–3,100], p < 0.001), and gestational age at delivery (34 weeks [IQR 31–37] vs 37 weeks [IQR 36–38], p < 0.001). There were no significant differences in colonisation proportions by sex (p = 0.7) or mode of delivery (p = 0.5; Table 1)

Overall, colonised neonates were significantly more likely to have been admitted to the NICU at any point during the study period (73% vs 45%, p < 0.001) and required longer NICU admissions than non-colonised neonates (median: 16 days [IQR 3–30] vs 2 days [IQR 2–7], p < 0.001). Our NICU admission variable served as a proxy that encompassed exposure to medical interventions routinely delivered during a NICU admission, such as the use of indwelling lines and mechanical ventilation, which may also predispose to MDR colonisation risk (further delineated in Supplementary Table 18) However, when analysing these differences by colonisation group, this difference was not significant in the CR-GN cohort (Table 1). Colonised neonates were also significantly more likely to have received antibiotics prior to their first positive swab (72 [63%] vs 8 [24%], p <0.001; Table 1). When analysed by antibiotic class, this significant association was evident following exposure to penicillins (14 (19%) vs 51 (44%), p <0.001) and aminoglycosides (18 (24%) vs 70 (61%), p <0.001) (Table 1, Supplementary Figure 4).

Maternal exposure to antibiotics in the antenatal period (p= 0.10) or during delivery (p=0.3) was not associated with a significantly increased risk of neonatal colonisation. However, when analysing antibiotic exposure by class, maternal exposure to cephalosporins during delivery was significantly more frequent among colonised neonates overall (57% vs 35% of non-colonised neonates, p = 0.003), and for neonates colonised with ESBL (56% vs 41%, p = 0.048) and CRE pathogens (59 (42%) vs 33 (66%), p = 0.004). However, no antibiotic classes were significantly associated with colonisation with other CR-GN pathogens (Table 1).

Longer hospital admission in any ward consistently increased the odds of colonisation with any MDR-GN bacteria, while NICU admission and neonatal antibiotic exposure emerged as additional predictors in LASSO-selected models for subsequent colonisation with MDR-GN. No significant predictors were identified for other CR-GN bacteria. The results of the LASSO regression models are summarised in Supplementary Tables 6-11.

Impact of neonatal colonisation on clinical outcomes:

Clinically suspected infection episodes (defined as clinical instability accompanied by the decision to commence anti-infective medication) occurred in almost half of all enrolled infants (47%, 89/189); with 19 of these infants (of 189, 10%) experiencing more than one infection episode during their hospital admission (Table 2). Neonates who were colonised at any timepoint during the study were observed to have more

frequent sepsis episodes, including both culture-negative and culture-positive events. Almost two thirds of colonised infants (71/115, 62%) suffered ≥1 sepsis episode (versus 24% of non-colonised neonates; $p < 0.001$; Table 2).

Eleven culture-positive sepsis episodes occurred, of which two were causative of early-onset disease whilst the remaining nine occurred at >48 hours of life, and seven were caused by the pathogens of interest in our study. The most frequently isolated pathogens in blood were *K. pneumoniae* (4/11, 36%) and *Staphylococcus epidermidis* (3/11, 27%), with *Klebsiella oxytoca* (n=1), *Acinetobacter baumannii* (n=1), *Serratia marcescens* (n=1) and *Staphylococcus aureus* (n=1) also identified as causative of infection (Figure 3, Supplementary Table 3).

Only one culture-positive neonatal sepsis event occurred with a pathogen phenotypically identical to a previously colonising pathogen (confirmed via bacterial identification and antimicrobial susceptibility testing). This episode of ESBL-producing *K. pneumoniae* bacteraemia occurred on day 11 of life, following previously-identified colonisation with the same pathogen on day 3, in a neonate born to a mother colonised with ESBL-producing *K. pneumoniae.* The antibiograms of these three isolates all aligned. Among the remaining ten infants with culture-positive sepsis episodes, no colonising pathogens phenotypically matched the invasive pathogen causative of infection.

In the overall mortality model, neonatal colonisation was associated with a higher odds of mortality (OR 4.35, 95% CI 1.12 to 17.60, $p = 0.030$), when adjusted for other variables. Hospital admission duration was also associated with a higher odds of mortality (1.10, 95% CI 1.02 to 1.20, $p = 0.025$). Neonatal antibiotic exposure during hospital admission was inversely associated with overall mortality. ($p<0.001$; Table 3; Supplementary Tables 12-15).

Environmental Swabs:

Overall, 20 environmental swabs were collected from six different environmental sites, via fortnightly sampling. Resistant gram-negative bacteria were detected across all environmental sites where weekly swabbing was undertaken; including handwashing sinks, participant cots, hand sanitiser units, neonatal pulse oximeters, water reservoirs of humidifiers, and computer keyboards, with CR-GN predominating as the most frequently isolated resistance mechanism in environmental samples (Supplementary Table 16). The most frequently positive environmental swabbing site was a handwashing sink drain, with CR-GN isolated most frequently (40% of samples, 8/20). Almost half of the environmental swabs collected from keyboards yielded a positive culture (9/20). One in five pulse oximeter swabs yielded a gram-negative pathogen, with 15% of

samples ESBL-producing. The tops of hand sanitiser sprays, swabs collected from under cots, and humidifier water reservoirs revealed lower yields (all ≤10% for any organism). CRE was rarely detected in environmental samples; and was only isolated in handwashing sink drains (5% of samples).

Discussion

We conducted a detailed prospective surveillance study to delineate the role of vertical and horizontal acquisition of MDR gram-negative bacteria in neonates, and the impact colonisation has on subsequent episodes of sepsis and clinical outcomes. Our study revealed that neonates are rapidly colonised with MDR gram-negative bacteria following admission to the NICU, and that colonisation predisposes to a higher rate of subsequent infection episodes, and a higher odds of mortality. However, we did not find evidence that invasive infections are caused by the same pathogens identified as preceding colonising bacteria.

Our study has also identified a high prevalence of ESBL colonisation in mothers presenting for delivery, indicating the high community prevalence of this antibiotic resistance mechanism in Indonesia. However, carbapenem-resistant gram-negative colonisation was low in mothers presenting for delivery, yet rapidly acquired by most neonates admitted to the NICU, suggesting horizontal-acquisition of these resistance mechanisms predominates, aligning with the results of our environmental sampling.

Colonisation with MDR-GN bacteria in neonates prospectively followed in our study correlated with the likelihood of being admitted to the NICU, a longer NICU stay, and prior exposure to antibiotics. This suggests antibiotics may disrupt the neonatal microbiome and promote selection pressure of MDR bacteria. This was particularly prevalent for antibiotics that are not particularly broad-spectrum – namely, the penicillin and aminoglycoside classes; and is an important finding in reminding clinicians of the need to carefully consider the risks versus benefits of cautious empiric antibiotic prescribing in clinical scenarios where the true risk of sepsis may be low. Previous studies have identified the deleterious effect of antibiotics in promoting MDR bacterial selection and transmission,[29–31] and a clearer understanding of the role of various antibiotic classes in altering microbiome richness and diversity, and subsequent selection and transmission of antibiotic resistance mechanisms, is needed to elucidate this important association in neonatal colonisation and infection.

Our study demonstrated a correlation between neonates colonised with MDR-GN bacteria and poorer clinical outcomes, including more frequent infection episodes and a higher odds of mortality. It is difficult to delineate the role of colonisation versus other risk factors in contributing to poor clinical outcomes in neonates, given the inherent high-risk population they encompass, although the mortality odds remained increased in

colonised neonates even when other variables were adjusted for in our logistic regression analysis. To confirm this association, larger, multicentre prospective surveillance studies are needed to more clearly identify the risk of colonisation in driving adverse clinical outcomes in neonates.

We did not find colonisation with MDR-GN bacteria correlated closely with pathogens subsequently isolated in culture-positive invasive sepsis episodes, with only one infant (of 114 sepsis episodes, including 11 culture-positive infections) experiencing an invasive (bloodstream) infection with the same pathogen identified in a prior rectal swab. However, our small sample size constrains our ability to draw any definitive conclusions around the risk of invasive infections with colonising pathogens. Nevertheless, this has may have important implications for IPC strategies that currently promote cohorting patients with colonisation of similar antibiotic resistance mechanisms, and for the empirical prescribing of broad-spectrum therapy if a neonate known to be colonised with a bacterial pathogen deteriorates.

Our findings suggest empirical broad-spectrum therapy may not be indicated in these circumstances, as most infants with invasive infections had these caused by pathogens *not* previously identified in colonisation sampling. Overly broad-spectrum empiric antibiotic prescribing drives selection pressure in NICUs and propagates the transmission of antimicrobial-resistant genes. This should be a focus of consideration for future research, to promote narrow-spectrum prescribing and reduce the spread of AMR where possible. Clinicians should also remain astute in considering other pathogens that may be causative of invasive infection, despite prior colonisation results in unstable patients.

Our findings have revealed high colonisation rates, beyond those previously published in most settings. A recent meta-analysis evaluating MDR-GN colonisation in neonates estimated a pooled prevalence of approximately 30% for ESBL-producing and 3% for carbapenem-resistant bacteria across LMICs.[21] By contrast, our study revealed 61% of infants were colonised with MDR-GN bacteria by hospital discharge (and at a mean postnatal age of 10 days), and a CRE colonisation prevalence of 26% by discharge - almost ten-fold that reported in the literature. These findings likely reflect both the higher local burden of AMR in Indonesia,[12] and the rapidly growing global burden of MDR bacteria that are increasingly difficult to treat – particularly in neonates.[13] Indeed, other recent studies from resource-constrained healthcare settings have similarly reported very high colonisation rates – including ESBL-carriage of 86% in neonates in Cambodia,[22] and 85% carriage in the Gambia by 7 of life.[32] A Thai study screening infants younger than 28 days old at a large tertiary hospital reported a high prevalence of carbapenem-resistant GNB (38%) with *Acinetobacter* spp., now a major cause of neonatal sepsis globally, frequently identified.[27]

Maternal colonisation rates at delivery, particularly with ESBL-producing pathogens, were also higher in our cohort than has been reported in other studies, reflecting high community prevalence of these resistance mechanisms in Indonesia. Recent analyses suggest a pooled global prevalence of maternal colonisation with ESBL-producing *E.coli* at 34%, although though this has been estimated to be up to 50% in settings in Asia,[33] aligning with our findings (of 63% maternal ESBL-colonisation at delivery). However, maternal colonisation with CRE in our cohort was substantially lower than ESBL-colonisation (3%, 6/189) and was also lower than the overall prevalence in our enrolled neonates (26%, 50/189). These findings suggest that, in neonates, colonisation with carbapenem-resistant gram-negative is predominantly acquired from the hospital environment (including from other colonised patients), whereas ESBL-producing pathogens may be transmitted via both vertical or horizontal routes.

Our study revealed that selection pressure following antibiotic exposure likely plays a significant role in increasing the likelihood of CRE colonisation in hospitalised neonates. This is a key novel finding revealed by our cohort, as most published studies to-date have focussed on neonatal ESBL acquisition, confirming the importance of both vertical and horizontal transmission of this resistance mechanism.[28,34] However, our unique methodology that included ongoing surveillance swabbing of infants (even those ESBL-colonised) allowed capture of the high rates of subsequent carbapenem-resistance mechanism acquisition. The importance of prior antibiotic that was significantly associated with colonisation with ESBL and CREs highlights the role of antimicrobial selection pressure in driving MDR-GN colonisation.

By contrast, antenatal or perinatal maternal antibiotic exposure was not significantly associated with either maternal or infant MDR-GN colonisation (overall) in our cohort. However, when analysed by antibiotic class, maternal cephalosporin exposure in the perinatal period *did* influence neonatal acquisition of infant MDR-GN bacteria overall, as well as for ESBL-producers and CREs individually. This finding aligns with prior studies reporting that specific antibiotic classes may affect colonisation by pathogenic organisms and disrupt the developing infant microbiome.[32]

A meta-analysis of studies conducted in LMICs revealed neonatal antibiotic use is a significant risk factor for colonisation with ESBL-producing bacteria.[21] Microbiome studies suggest that at birth, the neonatal gut microbiome represents a low-diversity ecological niche that matures over the first three years of life, and use of antibiotics during this time may alter gut microbiota composition selecting for carriage of MDR-GNs,[34,35] providing biological plausibility for this risk factor. These findings highlight the importance of antimicrobial stewardship and appropriate prescribing in the neonatal period.

There are many reasons a NICU environment may predispose an infant to nosocomial colonisation with CREs, though perhaps most pressing is the selection pressure driven by high rates of empiric antibiotic use, which is common in NICUs.[20,36] Concurrently, antibiotic stewardship and IPC programs may be challenging to implement when facilities are crowded, or when resources are limited.[36,37] CRE 36,37acquisition dynamics behaved differently from ESBL acquisition in our cohort: in addition to a striking colonisation prevalence difference between mothers and infants (3% vs 26%), CRE colonisation tended to occur later than ESBL colonisation in neonates in our study, implying CRE colonisation is more likely to occur after extended exposure to the NICU environment.

In our study, admission to the NICU, and a longer NICU admission, were consistently associated with infant colonisation with MDR-GN pathogens - even after adjustment for potential confounders, reaffirming the role of the hospital environment in MDR-GN transmission. Similar associations have been observed in other resource-constrained healthcare settings, in India and Kenya.[18,24] However, CRE colonisation in these studies was not explored, as is a unique finding in our study. In our penalised analyses using LASSO regression, NICU admission was the only variable retained as a predictor of infant MDR-GN colonisation. This likely reflects the strong correlation between NICU admission and other clinical factors (such as low birthweight, prematurity, and antibiotic exposure), with LASSO preferentially selecting a single representative variable. Therefore, the exclusion of the other variables should not be interpreted as evidence that these factors are unimportant, but rather that they may lie along a shared clinical pathway.

Our study also demonstrated that colonised neonates have poorer clinical outcomes than non-colonised neonates, experiencing more subsequent sepsis episodes, and a higher overall risk of mortality. Whilst culture-positive infections were only evident in 9.6% of all sepsis episodes in our cohort (11/114), this aligns with prior studies[13] and confirms the challenges in identifying the pathogens causative of neonatal sepsis. In this study, we utilised the definition of sepsis that has been consistently applied in regional surveillance programs via the ACORN surveillance project ((https://acornamr.net/#/) to support a consistent epidemiological approach across international multicentre sepsis surveillance programs in light of the challenges in establishing a consensus definition for neonatal sepsis evident in the literature.[38] Many colonised infants may have developed invasive bacterial infections which were not detected (and classified as ‘culture negative’) due to the sub-optimal sensitivity of blood cultures and diagnostic challenges that are inherent to diagnosing neonatal sepsis (given the low blood volume frequently attained, and high rates of pre-treatment with antibiotics).[38] The observed inverse association between neonatal antibiotic exposure during hospital admission and overall mortality may be consistent with this hypothesis, as some infants with ‘culture negative’ infections may have received effective treatment with antibiotics that improved their clinical outcomes.

Several limitations exist for this study. Although we employed comprehensive maternal and infant gram-negative colonisation screening utilising selective agar and automated pathogen identification methods alongside phenotypic assays for AST, and utilised statistical analyses to characterise colonisation patterns and infer potential transmission dynamics, the unavailability of whole genome sequencing to more carefully map transmission pathways between mothers, babies and the hospital environment limits the interpretation of our findings. Nevertheless, we did identify the important finding of a matched antibiogram between a colonising *K. pneumoniae* isolate in a mother, their neonate (on day 3) and the invasive *K. pneumoniae* isolate that resulted in that neonate's death. Interestingly, LSCS was the most common mode of delivery in our study, reflecting the rapidly increasing rates of caesarean section delivery in Indonesia (and other settings in Southeast Asia) over recent years.[39,40] This may have resulted in lower rates of vertical transmission of MDR-GN pathogens than may occur in healthcare settings with lower rates of LSCS delivery, as exposure to bacterial flora during the birthing procedures may increase this perinatal transmission risk. However, delivery method was not identified as a significant risk factor influencing overall neonatal colonisation risk in our cohort.

Furthermore, we relied on the high reported sensitivity and specificity of CHROMagar plates in identifying drug-resistant bacteria, and did not have the resources to confirm these with further disc diffusion testing, although the automated AST results did concur these plate results. There were also challenges following up all the infants enrolled in our study, with 38 infants (15%) unable to be contacted at the 6-month follow-up point. This may underpower or bias or results relating to neonatal mortality. Additional limitations include the relatively small sample size, which may have reduced the statistical power to detect certain associations, alongside resource restrictions that required the culturing, identification, and AST processes to be limited to the most predominant growth evident on culture plates, potentially resulting in under-reporting of less abundant pathogens.

Nevertheless, our study provides key findings relating to the colonisation of MDR gram-negative bacteria in neonates, with a unique focus on both ESBL-producing and carbapenem-resistant mechanism acquisition, and reveals the environmental role of colonisation with MDR gram-negative bacteria in the NICU. This can promote the identification of opportunities to reduce this burden, particularly via ongoing environmental sampling. We have identified antibiotic prescribing as a risk factor for subsequent colonisation, particularly for carbapenem-resistant gram-negative bacteria, promoting the importance of antibiotic stewardship programs in NICUs. Our study also reveals the increasing, and high, community prevalence of ESBL-producing gram-negative bacteria.

Our findings are an important contribution to the literature, particularly given the high burden of AMR within the Asia region, where AMR increasingly predominates as a cause of neonatal mortality.[4,5,7] Recent studies from tertiary hospitals across Southeast Asia have demonstrated relatively consistent patterns of antimicrobial resistance and pathogen predominance in neonatal sepsis,[4–6,12] which may generalise our findings to other tertiary care settings in the region. While multiple factors contribute to MDR-GN neonatal colonisation, we have identified the importance of antibiotic stewardship and the hospital environment in contributing to colonisation with carbapenem-resistant pathogens; and identified the higher risk of subsequent sepsis episodes in these infants once colonised. However, our study also found colonisation with MDR-GN pathogens was not necessarily a predictor of subsequent invasive infection with the same pathogen, which should promote the rational use of empiric antibiotics that do not necessarily need to provide coverage against colonising bacteria when empirically treating neonatal sepsis. Future studies should evaluate the relationship between antibiotic exposure and CRE-colonisation more closely, analysing the impact of antibiotic exposure on the neonatal microbiome and utilising sequencing methods to map vertical and horizontal transmission pathways more closely. This will enable a greater focus to be given to measures that can promote the IPC and antimicrobial stewardship measures that will allow the rising burden of MDR-GN bacteria to be curtailed, to improve clinical outcomes for vulnerable newborn babies.

Methods

Ethics Statement

Ethical approval was provided by Universitas Indonesia (KET-1321/UN2.F1/ETIK/PPM.00.02/2023), and written informed consent was obtained from all maternal participants. Participant compensation for enrolment in the study was not provided.

Study design & location

We conducted a prospective cohort study between March to December 2024 at Cipto Mangukusomo hospital, a tertiary urban referral hospital in Jakarta, Indonesia. The 1,001-bed hospital has a delivery ward with 1,500 neonates delivered each year, alongside 66 NICU beds and two postnatal wards. Approximately 1,400 neonates are admitted to the NICU each year.

All pregnant women (>18 years) delivering at the hospital were invited to participate in the study and were enrolled if they provided informed consent prior to delivery. Mother-infant dyads were excluded from the study if a stillbirth occurred, or if the infant was born with an imperforate anus (due to planned rectal swabs). The required sample size of 189 was calculated based on an estimated median time-to-event ESBL colonisation of seven days, with a desired precision of +/- 1 day assuming an exponential survival distribution using the formula $z = \left(\frac{Z_\alpha/2 \times \sigma)}{err}\right)^2$ where $Z_\alpha/2$ is the normal distribution, σ is the exponential standard-deviation for time-to-event data, and err is the precision.

Specimen collection and laboratory methods

Rectal swabs (Copan FLOQswabs in amies medium) were collected from mother-infant dyads to assess colonisation with drug-resistant gram-negative bacteria. Rectovaginal swabs were collected from mothers within 15 minutes of delivery; and rectal swabs were collected from infants within 15 minutes of delivery, then on admission to the NICU, and subsequently every 72 hours until day 14. Subsequently, swabs were collected weekly from infants admitted to the NICU. A final rectal swab was collected from all neonates on discharge from the NICU or postnatal ward (Supplementary Figure 2). Environmental sampling was conducted fortnightly from high touch and wet areas across the NICU.

Swabs were inoculated onto chromogenic media (CHROMagar™ ESBL and CHROMagar™ KPC) to screen for drug-resistant gram-negative bacteria and incubated for 48 hours. If growth occurred, the primary pathogen evident were defined as extended-spectrum beta-lactamase (ESBL)-producing (on the CHROMagar™ ESBL plate) and/or carbapenem-resistant (CR; if growth occurred on the CHROMagar™ KPC plates), due to the high sensitivity and specificity of this growth aligning with the presence of these resistance mechanisms, as

reported in the literature.[31,41,42] Further supportive testing was conducted on the predominant colony from each plate via matrix-assisted laser ionisation-time of flight (MALDI-ToF) and automated antimicrobial susceptibility testing (AST) utilising the Phoenix™ Becton, Dickinson and Company [BD] automated system. Antimicrobial susceptibility profiles were reported using the Clinical & Laboratory Standards Institute (CLSI) standards (version 2024).[43]

Data collection:

Detailed demographic and clinical data were collected from mothers and infants to evaluate household circumstances (including crowding and access to water, sanitation and hygiene measures), maternal baseline health, and any treatments or interventions received during pregnancy (including exposure to any antibiotics; Supplementary Table 18). Enrolled neonates were reviewed daily throughout their hospital stay and followed-up for six months from the date of enrolment. At birth, neonatal clinical outcome data were recorded, and any medical interventions required (including exposure to antibiotics) and laboratory tests conducted were recorded. Any episodes of sepsis – defined as clinical instability accompanied by the decision to start antibiotic or antifungal therapy – that occurred during the neonatal hospital admission were recorded. Culture-positive infection episodes were also recorded, including the pathogen causative of infection (Supplementary Table 17). Clinical outcomes were ascertained via follow-up assessments conducted at day 28, and 3 and 6 months after enrolment (conducted via either clinic visits or phone calls, where routine clinical appointments were not required). All data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at the University of Sydney.

Statistical analysis

Statistical analyses were carried out using R (version 4.4.3). Summary statistics were generated separately for infants and mothers, and stratified by colonisation status across four categories: (i) colonisation with any gram-negative bacteria; (ii) colonisation with ESBL-producing gram-negative bacteria; (iii) colonisation with carbapenem-resistant Enterobacterales (CRE), and (iv) colonisation with other carbapenem-resistant (non-fermenting) gram-negative bacterial pathogens (CR-GN, ie, *Acinetobacter* spp. or *Pseudomonas aeruginosa*). Supplementary table 17 summarises the included pathogens, following exclusion of colonising bacteria, aligning with the definitions utilised in the neonatal sepsis literature globally.[3,5]

Variables were summarised using medians and interquartile ranges (IQRs), and categorical variables using frequencies and percentages. To compare between groups, Pearson's Chi-squared test or Fisher's exact test were used for categorical variables (depending on expected cell counts). Wilcoxon rank-sum tests were used for continuous variables. All hypothesis tests were two-sided, with $p<0.05$ considered stcatistically significant.

Given the exploratory nature of the analyses, formal adjustment for multiple comparisons was not performed. In the summary tables, we used gtsummary (tbl_summary with missing="no" and add_p()) functions, which excludes missing values from descriptive statistics and p-value calculations. Binary, multi-variable, and logistic regression models were constructed to examine predictors of neonatal colonisation and neonatal mortality. Separate models were constructed for colonisation with any organism, and colonisation with ESBL, CRE or CR-GN bacteria. Candidate covariates evaluated in regression models are summarised in Supplementary Table 18, and were pre-selected based on clinical expertise with combined input from study investigators and correlation analysis. Continuous variable Pearson correlation coefficients, and categorical Cramér's V statistics were calculated for correlation analysis. We analysed pairwise correlation between variables with a plausible correlation and excluded one of the variables if high correlation was found. Multivariable analyses included all participants with complete covariate and outcome data for the specified model.

To confirm robustness of variable selection, Least Absolute Shrinkage and Selection Operator (LASSO) regression models were performed for each combination of colonisation category and outcome. LASSO regression was used as a penalisation approach to highlight the key predictive variables for the various outcomes modelled, by shrinking less informative coefficients towards zero and retaining those with the strongest independent predictive contribution. Optimal penalty parameter (lambda) was chosen via cross-validation. Models were then re-fit using variables selected by LASSO. For regression models (glm with binomial link), analyses used complete-case (listwise deletion) by default (na.omit); and in the LASSO pipeline we explicitly applied na.omit() to the model dataset before fitting.

Time-to-event analyses were performed to estimate the time from birth to first detsection of colonisation for any organism; and separately for ESBL, CRE, and CR-GN bacterial colonisation. Infants without a positive neonatal swab ('event') at each sampling time point were censored at the final sampling date. We estimated colonisation-free survival functions using Kaplan–Meier methods and displayed cumulative incidence as $1-S(t)$. To aid presentation, we tabulated cumulative numbers colonised at 5-day intervals and reported the mean observed time-to-colonisation among infants with events. Kaplan–Meier curves and log-rank tests were computed using the *survival* package in R, with curves visualised with *survminer*. For subgroup analyses, we generated stratified Kaplan–Meier curves by prespecified covariates (such as admission to NICU) and compared strata using two-sided log-rank tests. Adjusted Cox proportional hazard models were then used to adjust for covariates in time-to-event analyses.

Data Availability

The data that support the findings of this study are not openly available due to reasons of sensitivity. However, data are available from the corresponding author upon reasonable request and with permission from the research and governance committee of Universitas Indonesia (KET-1321/UN2.F1/ETIK/PPM.00.02/2023).

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Author Contribution Statement

PCMW, BD, NDP: Conceptualisation, methodology, funding acquisition; Writing – review and editing. PT, AHJ: methodology, Writing-review and editing. PCMW, JH, JR, MH, CG: Formal analysis; Writing – original draft, Visualisation, Writing – review and editing. DCL, WT, RI, ATPI, AR, ASZ, MSV, MH: Investigation, resources, Data curation, Project administration, Writing – review & editing.

Competing Interest Statement

PCMW is supported by a National Health and Medical Research Council (NHMRC) Fellowship. The study was supported by funding from The Avant Foundation (BD), Sydney Institute for Infectious Diseases (Sydney ID), and Wellcome Trust (Grant number 222156/Z/20/Z, PT). The funders had had no role in the design, implementation or analysis of the study findings. All other authors declare no competing interests.

TABLES

Table 1.1 *Risk factors for (a) maternal and (b) neonatal colonisation.*

(a) Risk Factors for maternal colonisation. Stratified by any colonisation (total), ESBL-producing bacterial colonisation, carbapenem-resistant Enterobacterale colonisation (CRE), or carbapenem-resistant gram-negative (non-fermenting) bacterial colonisation (including Acinetobacter spp. and Pseudomonas spp.; CR-GN). Note: P-values were calculated using two-sided Wilcoxon rank-sum tests for continuous variables and Pearson's Chi-squared or Fisher's exact tests for categorical variables, as appropriate. Rows in **bold** highlight (statistically) significant findings.

Factor	Total Colonised			ESBL			CRE			CR-GN		
	Not-colonised (69, 36%)	Colonised (120, 63%)	p-value	Not-colonised (86, 46%)	Colonised (103, 54%)	p-value	Not-colonised (183, 97%)	Colonised (6, 3%)	p-value	Not-colonised (156, 82%)	Colonised (33, 17%)	p-value
Maternal Age (median, IQR)	31.0 (27.7, 35.7)	31.5 (26.2, 35.4)	>0.9	31.1 (27.7, 35.6)	31.5 (26.1, 35.5)	0.9	31.1 (26.4, 35.6)	30.8 (27.6, 34.3)	>0.9	31.1 (26.7, 35.3)	33.1 (26.3, 36.2)	0.3
Household Population ≦3:	22 (34%)	35 (32%)	0.8	26 (32%)	31 (33%)	>0.9	56 (33%)	1 (20%)	0.7	50 (35%)	7 (21%)	0.3
4 – 6 people	39 (61%)	72 (65%)		52 (64%)	59 (63%)		107 (63%)	4 (80%)		86 (61%)	25 (76%)	
≧7 people	3 (5%)	4 (4%)		3 (3.7%)	4 (4%)		7 (4%)	0 (0%)		6 (4.2%)	1 (3%)	
Hospital admission during pregnancy:												
Admitted	15 (22%)	34 (28%)	0.5	17 (20%)	32 (31%)	0.2	48 (26%)	1 (17%)	>0.9	43 (28%)	6 (18%)	0.2
Not admitted	52 (75%)	80 (67%)		65 (76%)	67 (65%)		127 (69%)	5 (83%)		108 (69%)	24 (73%)	
Unknown	2 (3%)	6 (5.0%)		4 (5%)	4 (4%)		8 (4.4%)	0 (0%)		5 (3.2%)	3 (9%)	
Antibiotics prescribed during pregnancy:												
Yes	9 (13%)	15 (13%)	0.047	9 (10%)	15 (15%)	0.2	23 (13%)	1 (17%)	0.7	23 (15%)	1 (3%)	0.078
No	60 (87%)	95 (80%)		75 (87%)	80 (78%)		150 (82%)	5 (83%)		127 (81%)	28 (88%)	
Unknown	0 (0%)	9 (7.6%)		2 (2.3%)	7 (6.9%)		9 (4.9%)	0 (0%)		6 (3.8%)	3 (9%)	
If antibiotics were prescribed during pregnancy, effect of different classes:												
Penicillins	0 (0%)	2 (2%)	0.5	0 (0%)	2 (2%)	0.5	2 (1%)	0 (0%)	>0.9	2 (1%)	0 (0%)	>0.9
Cephalosporins	4 (6%)	6 (5%)	>0.9	4 (5%)	6 (6%)	0.8	10 (6%)	0 (0%)	>0.9	9 (6%)	1 (3%)	>0.9
Azithromycin	3 (4%)	0 (0%)	0.047	3 (4%)	0 (0%)	0.092	3 (2%)	0 (0%)	>0.9	3 (2%)	0 (0%)	>0.9
Metronidazole	0 (0%)	2 (2%)	0.5	0 (0%)	2 (2%)	0.5	2 (1%)	0 (0%)	>0.9	2 (1%)	0 (0%)	>0.9
Ciprofloxacin	0 (0%)	1 (1%)	>0.9	0 (0%)	1 (1%)	>0.9	1 (1%)	0 (0%)	>0.9	0 (0%)	1 (3%)	0.2
Unknown class	4 (6%)	7 (6%)	>0.9	4 (5%)	7 (7%)	0.5	10 (6%)	1 (1%)	0.3	11 (7%)	0 (0%)	0.2

b) Risk factors for Neonatal colonisation by the total colonised (by an ESBL-producing bacteria (ESBL), carbapenem-resistant Enterobacterales (CRE) or carbapenem-resistant gram-negative (non-fermenting) bacteria (including *Acinetobacter spp*. and *Pseudomonas spp*.) (CR-GN)), ESBL, CRE and CR-GN.

Variable	**Total Colonised**			**ESBL**			**CRE**			**CR-GN**		
	Not-colonised (74, 39%)	Colonised (115, 61%)	p-value	Not-colonised (92, 49%)	Colonised (97, 51%)	p-value	Not-colonised (139, 73%)	Colonised (50, 26%)	p-value	Not-colonised (159, 84%)	Colonised (30, 16%)	p-value
Demographics & Birth Factors												
Sex (n = female)	40 (54%)	59 (51%)	0.7	40 (54%)	59 (51%)	0.7	76 (55%)	23 (46%)	0.3	81 (51%)	18 (60%)	0.4
Birthweight (grams; median)	**2,595**	**2,035**	**<0.001***	**2,595**	**2,035**	**<0.001***	**2,530**	**1,798**	**<0.001***	2,290	2,598	0.5
Gestational age (weeks; median) Range: 24-41 weeks	**37**	**34**	**<0.001***	**37**	**34**	**<0.001***	**37**	**34**	**<0.001***	37	36	0.4
Delivery method: LSCS	51 (69%)	85 (74%)	0.5	65 (71%)	71 (73%)	0.7	95 (68%)	41 (82%)	0.065	112 (70%)	24 (80%)	0.3
Delivery method: Vaginal	23 (31%)	30 (26%)		27 (29%)	26 (27%)		44 (32%)	9 (18%)		47 (30%)	6 (20%)	
Neonatal Intensive Care Unit (NICU) Admission												
NICU admission required	**33 (45%)**	**84 (73%)**	**<0.001***	**42 (46%)**	**75 (77%)**	**<0.001***	**73 (53%)**	**44 (88%)**	**<0.001***	100 (63%)	17 (57%)	0.5
NICU admission duration (days; median, range)	**2 (2, 7)**	**16 (3, 30)**	**<0.001***	**3 (2, 8)**	**16 (3, 30)**	**<0.001***	**3 (2, 18)**	**23 (8, 40)**	**<0.001***	5 (2, 23)	7 (2, 27)	0.7
Maternal antibiotic exposure												
Maternal antibiotic exposure during pregnancy (any antibiotic)	5 (6.8%)	19 (17%)	0.10	8 (8.8%)	16 (16%)	0.3	14 (10%)	10 (20%)	0.046	21 (13%)	3 (10%)	0.5
Maternal antibiotic exposure during delivery (any antibiotic)	47 (64%)	81 (70%)	0.3	61 (66%)	67 (69%)	0.7	89 (64%)	39 (78%)	0.070	104 (65%)	24 (80%)	0.12
Maternal antibiotic exposure during delivery (cephalosporins)	**26 (35%)**	**66 (57%)**	**0.003***	**38 (41%)**	**54 (56%)**	**0.048***	**59 (42%)**	**33 (66%)**	**0.004***	73 (46%)	19 (63%)	0.08
Neonatal antibiotic exposure prior to first positive colonising swab												
Neonatal exposure to antibiotics prior to first positive screening swab (any antibiotic)	**8 (24%)**	**72 (63%)**	**<0.001***	**25 (27%)**	**65 (67%)**	**<0.001***	**50 (36%)**	**40 (78%)**	**<0.001***	76 (48%)	14 (48%)	>0.9
Neonatal exposure to antibiotics prior to first positive screening swab: (penicillins)	**14 (19%)**	**51 (44%)**	**<0.001***	**18 (20%)**	**47 (48%)**	**<0.001***	**37 (27%)**	**28 (55%)**	**<0.001***	53 (33%)	12 (41%)	>0.9
Neonatal exposure to antibiotics prior to first positive screening swab: (aminoglycosides)	**18 (24%)**	**70 (61%)**	**<0.001***	**25 (27%)**	**63 (65%)**	**<0.001**	**50 (36%)**	**38 (75%)**	**<0.001***	74 (46%)	14 (48%)	0.8

Table 2: Neonatal outcomes: infection episodes (culture-positive and culture-negative) occurring in enrolled neonates across the study period, and mortality at follow-up by the total colonised (by an ESBL-producing bacteria (ESBL), carbapenem-resistant Enterobacterales (CRE) or carbapenem-resistant gram-negative (non-fermenting) bacteria (including *Acinetobacter spp*. and *Pseudomonas spp*.) (CR-GN)), ESBL, CRE and CR-GN

Number of infection episodes	**Total Colonised**			**ESBL**			**CRE**			**CR-GN**		
	Not-colonised	**Colonised**	**p-value**	**Not-colonised**	**Colonised**	**p-value**	**Not-colonised**	**Colonised**	**p-value**	**Not-colonised**	**Colonised**	**p-value**
No infection episodes	**56 (76%)**	**44 (38%)**	**<0.001***	**67 (73%)**	**33 (34%)**	**<0.001***	**90 (65%)**	**10 (20%)**	**<0.001***	84 (53%)	16 (53%)	>0.9
One	**16 (22%)**	**54 (47%)**		**21 (23%)**	**49 (51%)**		**40 (29%)**	**30 (60%)**		59 (37%)	11 (37%)	
Two	**2 (2.7%)**	**12 (10%)**		**4 (4.3%)**	**10 (10%)**		**7 (5.0%)**	**7 (14%)**		12 (7.5%)	2 (6.7%)	
Three	**0 (0%)**	**4 (3.5%)**		**0 (0%)**	**4 (4.1%)**		**2 (1.4%)**	**2 (4.0%)**		3 (1.9%)	1 (3.3%)	
Four	**0 (0%)**	**1 (0.9%)**		**0 (0%)**	**1 (1.0%)**		**0 (0%)**	**1 (2.0%)**		1 (0.6%)	0 (0%)	
Alive at last follow-up^												
Alive	54 (86%)	77 (84%)	0.7	67 (87%)	64 (82%)	0.4	105 (88%)	26 (74%)	0.057	108 (83%)	23 (92%)	0.4

Note: *Rows in **bold** highlight (statistically) significant findings.
^Alive at last available follow-up data point for individual

Table 3: Risk factors associated with an increased odds of mortality, following multivariable logistic regression.

	Odds Ratio (95% CI)	Standard Error	Statistic	p-value
Neonate colonised an ESBL-producing or carbapenem-resistant gram-negative pathogen	**4.4 (1.2 to 17.6)**	**0.7**	**2.2**	**0.03***
NICU admission required	0.296 (0.1 to 7.8)	1.45	-0.8	0.4
Hospital admission duration (per day)	**1.1 (1.0 to 1.2)**	**0.1**	**2.2**	**0.025***
Maternal antibiotics in pregnancy	2.6 (0.4 to 52.6)	1.2	0.8	0.412
Neonatal antibiotic exposure during admission	**0.1 (0.1 to 0.2)**	**1.2**	**-3.2**	**0.001***

Note: Odds ratios were estimated using multivariable logistic regression and are presented with 95% confidence intervals. *Rows in **bold** highlight (statistically) significant findings.

Figure Legends/Captions

Figure 1: Overall proportion of maternal and infant colonisation with gram-negative bacteria, by resistance mechanism type.

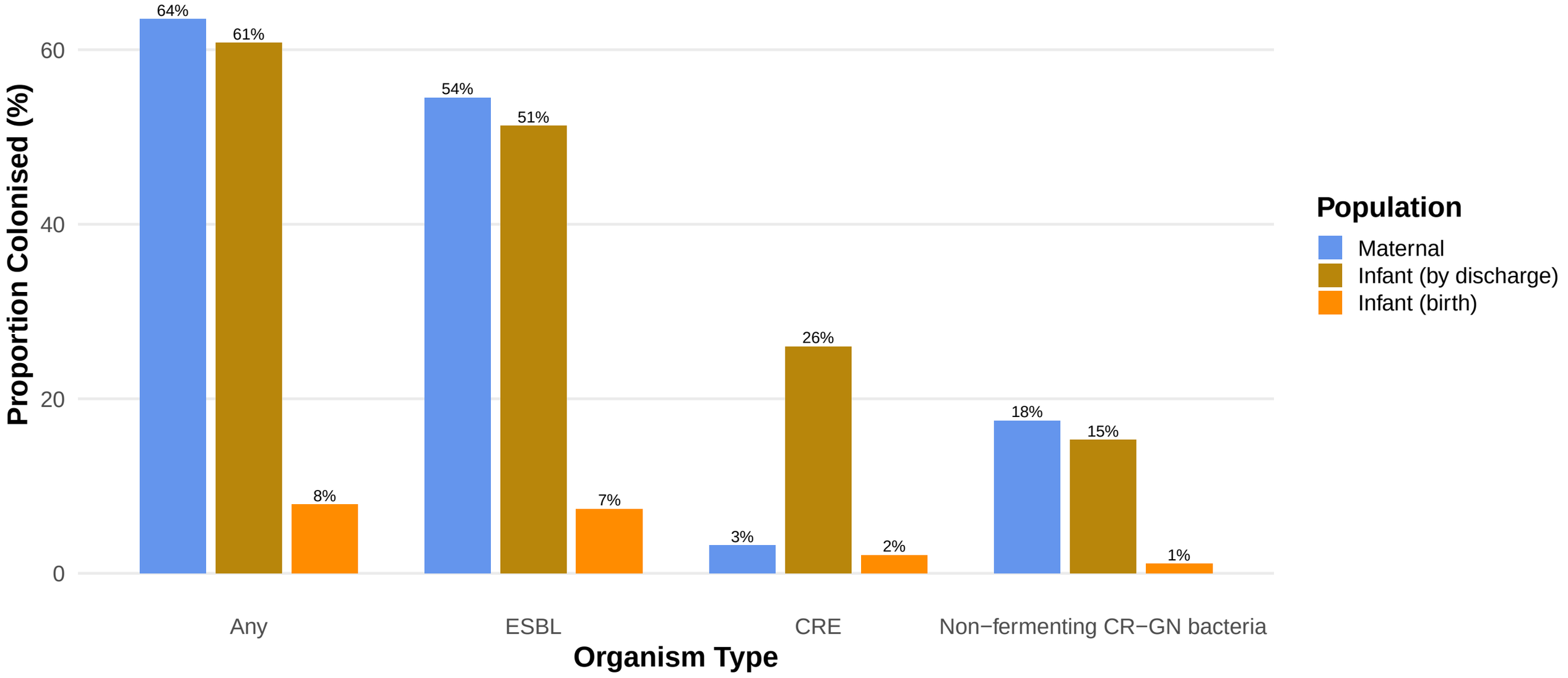
Note: ESBL = extended-spectrum beta-lactamase producing; CRE = carbapenem-resistant Enterobacterales; CR-GN = carbapenem-resistant non-fermenting bacteria (*Acinetobacter* spp., *Pseudomonas* spp.)

Figure 2: Kaplan–Meier curves summarising the cumulative time to neonatal colonisation with a. any resistant gram-negative bacteria, b. ESBL-producing bacteria, c. carbapenem-resistant Enterobacterales, and d. carbapenem-resistant gram-negative (non-fermenting) bacteria (including *Acinetobacter* spp. and *Pseudomonas* spp.)

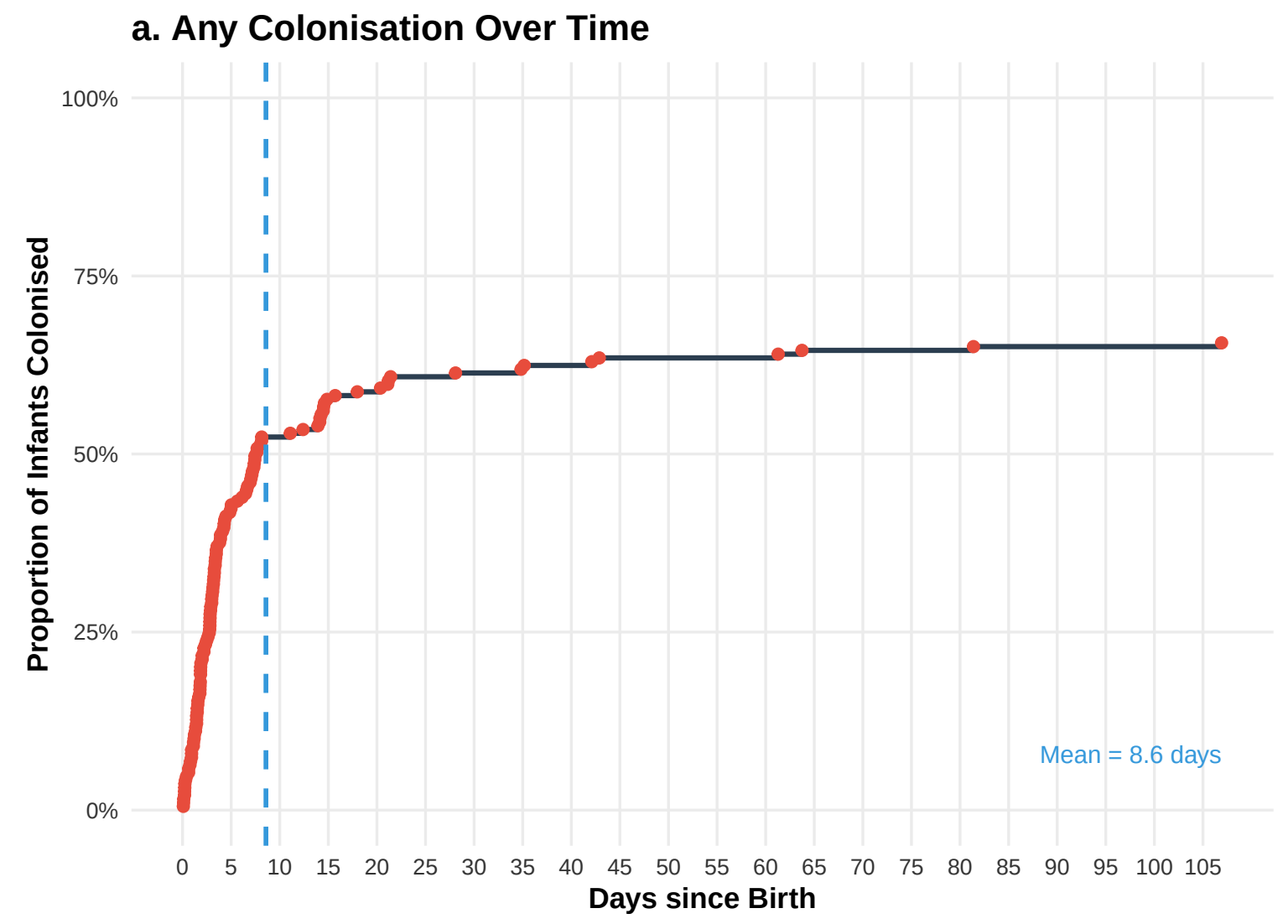
Note: Time-to-colonisation analyses were performed using Kaplan–Meier methods, with cumulative incidence displayed as 1−S(t). Infants without detected colonisation were censored at the time of their final screening swab. Curves were compared using two-sided log-rank tests. Adjusted Cox proportional hazards models were used for covariate-adjusted time-to-event analyses, as described in the Methods.

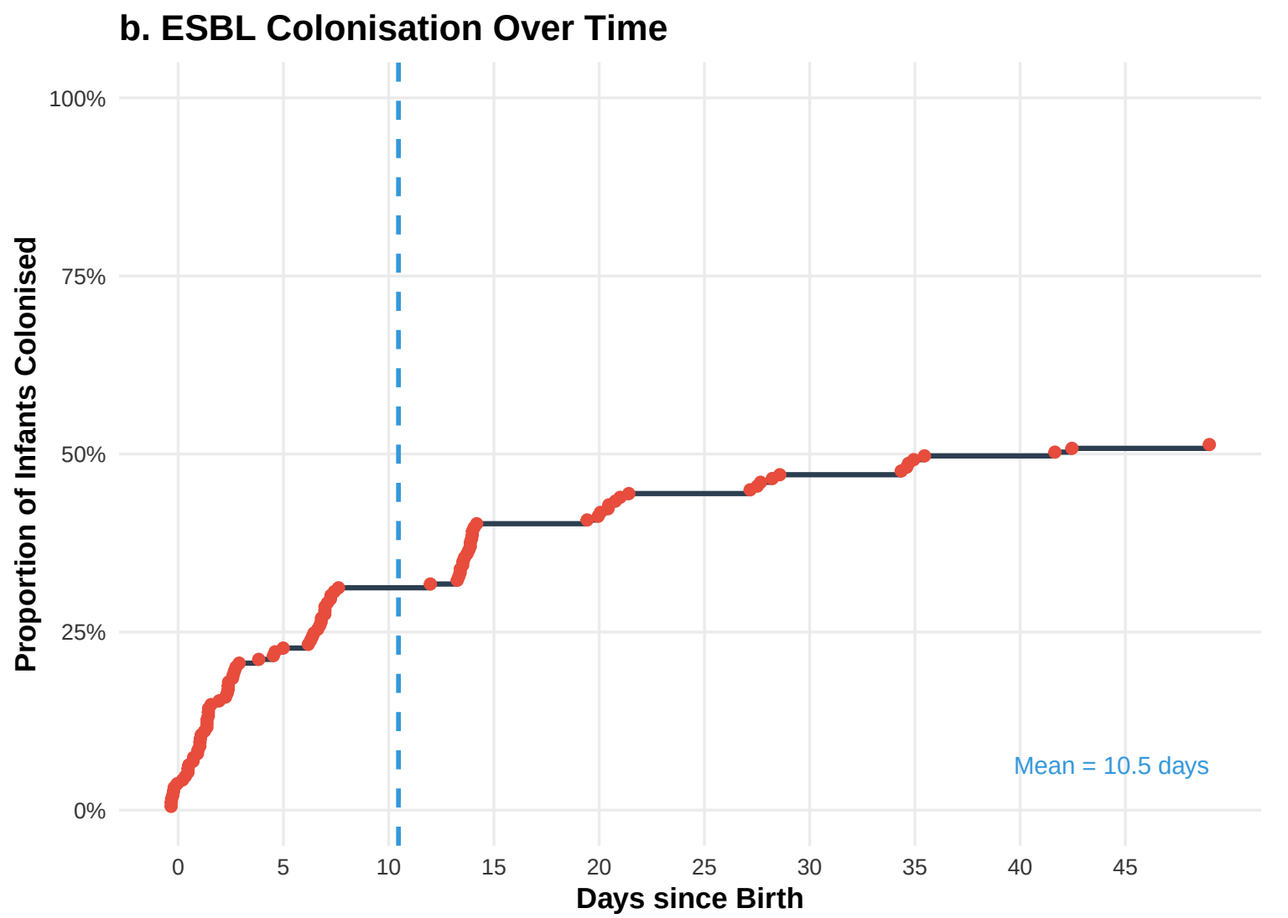
ESBL = extended-spectrum beta-lactamase-producing; CRE = carbapenem-resistant Enterobacterales; CR-GN = carbapenem-resistant non-fermenting gram-negative bacteria.

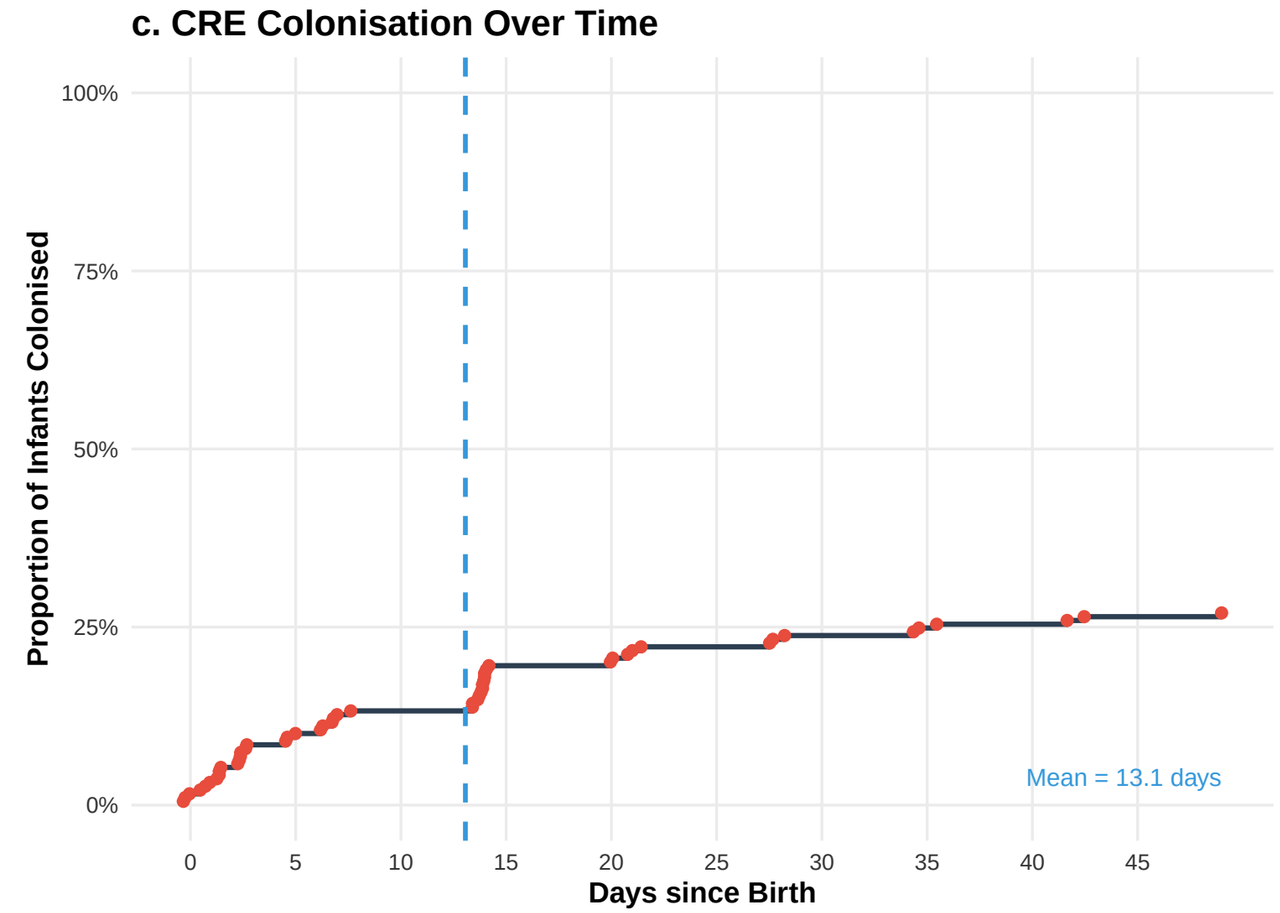
Figure 3: Frequency distribution of bacterial pathogens most frequently isolated (a) as colonising bacteria in mother-infant dyads and (b) as causative of invasive infections.

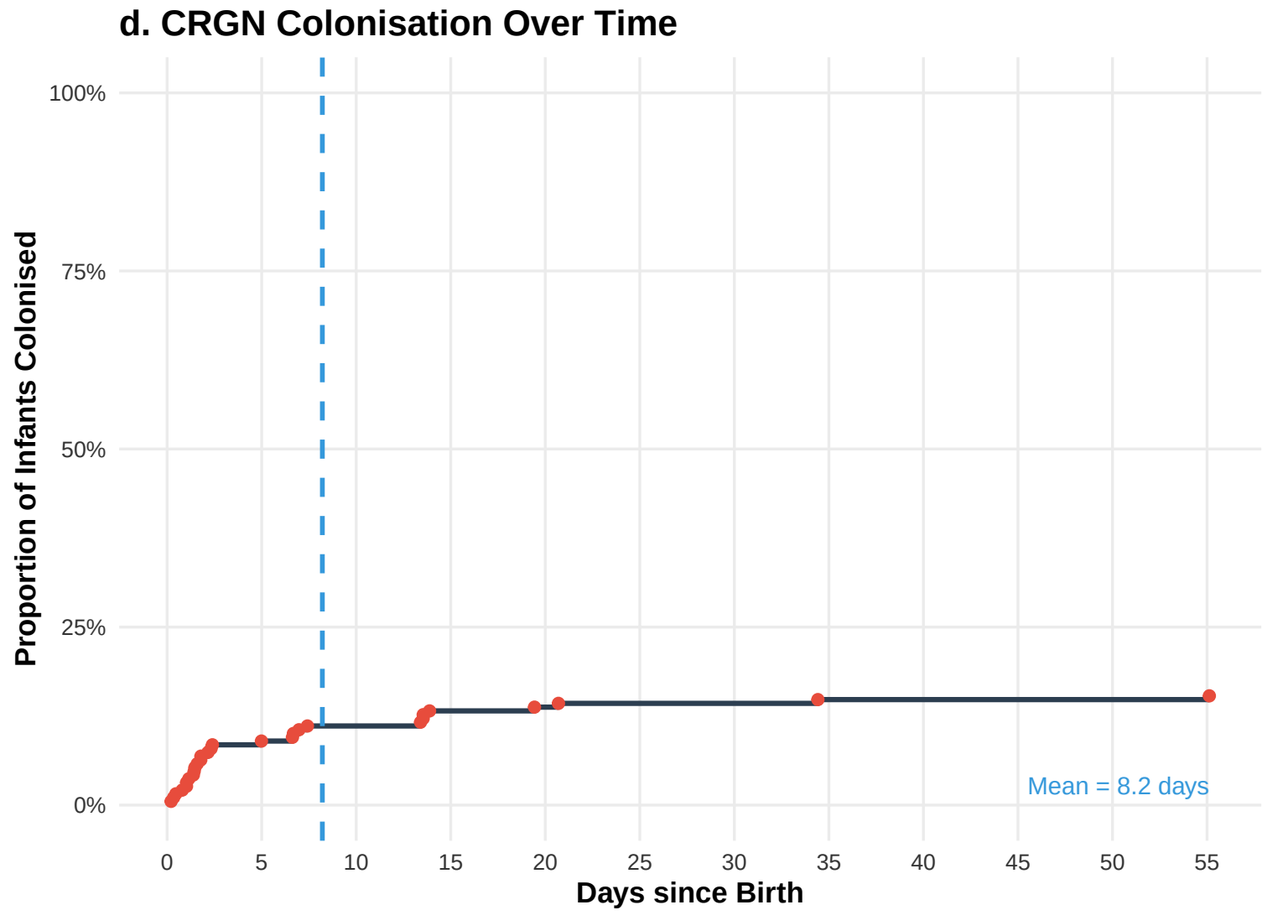

Proportion Colonised (%)
60
40
20
0
64%
61%
8%
54%
51%
7%
3%
26%
2%
18%
15%
1%
Any
ESBL
CRE
Non−fermenting CR−GN bacteria
Organism Type
Population
Maternal
Infant (by discharge)
Infant (birth)

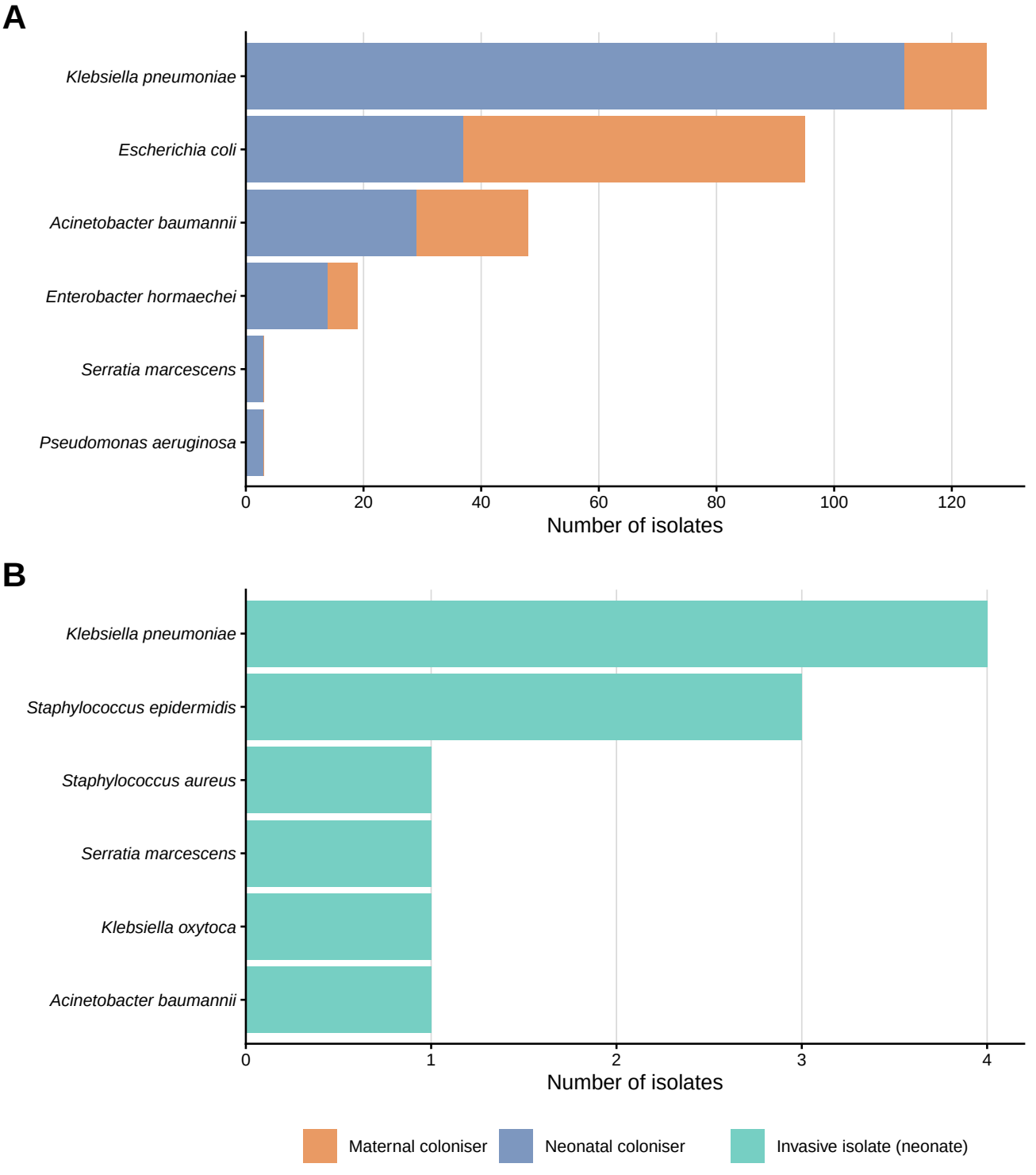
Cumulative Colonisation Over Time by Organism Type










A
Klebsiella pneumoniae
Escherichia coli
Acinetobacter baumannii
Enterobacter hormaechei
Serratia marcescens
Pseudomonas aeruginosa
0
20
40
60
80
100
120
Number of isolates
B
Klebsiella pneumoniae
Staphylococcus epidermidis
Staphylococcus aureus
Serratia marcescens
Klebsiella oxytoca
Acinetobacter baumannii
0
1
2
3
4
Number of isolates
Maternal coloniser
Neonatal coloniser
Invasive isolate (neonate)