# Etiology, Antibiotic Resistance and Risk Factors for Neonatal Sepsis in a Large Referral Center in Zambia

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**Background:** In sub-Saharan Africa, there is scanty data on the causes of neonatal sepsis and antimicrobial resistance among common invasive pathogens that might guide policy and practice.

**Methods:** A cross-sectional observational prevalence and etiology study of neonates with suspected sepsis admitted to the neonatal intensive care unit, University Teaching Hospital, Lusaka, Zambia, between October 2013 and May 2014. Data from blood cultures and phenotypic antibiotic susceptibility testing were compared with multivariate analysis of risk factors for neonatal sepsis.

**Results:** Of 313 neonates with suspected sepsis, 54% (170/313) were male; 20% (62/313) were born to HIV-positive mothers; 33% (103/313) had positive blood cultures, of which 85% (88/103) were early-onset sepsis. *Klebsiella* species was the most prevalent isolate, accounting for 75% (77/103) of cases, followed by coagulase-negative staphylococci [6% (7/103)], *Staphylococcus aureus* [6% (6/103)], *Escherichia coli* [5% (5/103)] and *Candida* species [5% (5/103)]. For *Klebsiella* species, antibiotic resistance ranged from 96%–99% for World Health Organization-recommended first-line therapy (gentamicin and ampicillin/penicillin) to 94%–97% for third-generation cephalosporins. The prevalence of culture-confirmed sepsis increased from 0 to 39% during the period December 2013 to March 2014, during which time mortality increased 29%–47%; 93% (14/15) of late-onset sepsis and 82% (37/45) of early-onset sepsis aged 4–7 days were admitted >2 days before the onset of symptoms. Culture results for only 25% (26/103) of cases were available before discharge or death. Maternal HIV infection was associated with a reduced risk of neonatal sepsis [odds ratio, 0.46 (0.23–0.93); $P = 0.029$].

**Conclusions:** Outbreaks of nosocomial multiantibiotic-resistant infections are an important cause of neonatal sepsis and associated mortality. Reduced risk of neonatal sepsis associated with maternal HIV infection is counterintuitive and requires further investigation.

**Key Words:** neonatal sepsis, Africa, HIV exposed, antimicrobial resistance, *Klebsiella pneumoniae*

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Epidemiological estimates suggest that there were 1.7 million cases of neonatal sepsis globally in 2010, with 0.6 million cases[1] and 0.14 million deaths[2] in sub-Saharan Africa.[2] In the World Health Organization (WHO) Africa region, the importance of neonatal infections and their contribution to under 5-year mortality is of concern.[2] Apart from the impact of sepsis on morbidity and mortality in neonates, and associated health system costs, there are also long-term developmental effects.[3] Improved understanding of the underlying causes of neonatal sepsis is required to better inform on management and prevention guidelines.

Thirteen neonatal sepsis studies from Africa published between 2010 and 2015[4–16] do not distinguish clearly between community-acquired or hospital-acquired neonatal sepsis and/or between vertically or horizontally acquired infections. *Klebsiella* species were commonly identified in all but one study, accounting for 32% (323/1009, range 0–59%). *Staphylococcus aureus* (24%) and coagulase-negative staphylococci (12%) were the second and third most prevalent organisms. These findings were similar to those reported in a review of 6 studies of hospital-acquired neonatal sepsis published between 1990 and 2004, where *Klebsiella* species were found in 28% (441/1563) of cases, followed by *S. aureus* (14.3%), *Escherichia coli* (9.9%), other Gram negatives (8.8%) and group B streptococci (GBS) (8.5%).[17]

The WHO recently declared antimicrobial resistance (AMR), a global health security threat because of the very high AMR rates globally, and highlighted the large gaps in surveillance.[18] With respect to neonates, available data demonstrate widespread resistance to first- and second-line therapy, for both community-acquired[19–21] and hospital-acquired[17,21,22] neonatal sepsis. The primary cause of resistance is plasmid-driven spread of extended spectrum beta-lactamases (ESBLs), which are common in Africa and confer resistance to all first- and second-line antibiotics.[23] There is also growing resistance to carbapenems in Africa.[24]

We report blood culture findings from an 8-month cross-sectional prevalence and etiology study of neonates with suspected sepsis admitted to the University Teaching Hospital, Lusaka, Zambia.

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## METHODS

### Ethics Approval

The study was approved by the Biomedical Research Ethics Committee of the University of Zambia School of Medicine (UNZABREC), Lusaka, Zambia. The mothers/guardians of all participants gave written informed consent.

### Study Design and Setting

We conducted a cross-sectional study on the neonatal intensive care unit (NICU) of the University Teaching Hospital, Lusaka, Zambia, over a period of 8 months (October 2013 and May 2014) with the primary aim of defining bacterial etiology of neonatal sepsis and determining AMR patterns. Secondary aims were to evaluate possible risk factors associated with culture-positive sepsis and its impact on mortality. In Lusaka district, an average of 660 low-risk deliveries per week took place in district midwifery-led delivery centers, 420/week at the referral center [University Teaching Hospital (UTH)], 77/week at the district hospital and about 79/week deliver at home. At UTH, approximately 80% of neonatal admissions are in-born, with 20% being referred from the community (Y.A., unpublished data). The neonatal unit at UTH accounts for 99% of neonatal deaths within Lusaka and surrounding towns, a population of approximately 2 million people. In the 4 months before study initiation, mortality was consistently above 50%, with an average monthly admission rate of 296 babies. Key descriptives of the recruited neonates were compared with this register to evaluate recruitment bias. The hospital employs WHO-recommended Option B+ for the prevention of mother-to-child transmission (MTCT),[25] so all neonates born to HIV-infected mothers are on daily nevirapine until 6 weeks of age and mothers are on antiretroviral therapy for life.[26] Where we use the term "HIV-exposed," we refer to all neonates born to HIV-infected mothers. HIV reverse transcriptase polymerase chain reaction is not routinely performed at birth and was not performed on this study.

### Participants

Inclusion criteria for neonates was "suspected sepsis," as defined by elevated temperature (>37.5°C) odds ratio (OR) elevated respiratory rate (50< breaths/minute). This definition was based on the local NICU protocol that captures the maximal number of cases. All neonates had a thorough clinical evaluation and donated blood for microbiologic analysis. We define early-onset sepsis (EOS) as "positive culture in neonates aged ≤7 days" (segregating into those aged ≤3 days and 4–7 days) and late-onset sepsis (LOS) as "positive culture in neonates aged >7 days."

### Microbiological Diagnosis

About 1.5 mL blood was collected by a dedicated neonatal phlebotomist, trained by Becton Dickinson (Franklin Lakes, NJ) on aseptic blood collection for culture. Samples were cultured using the BACTEC FX200 system (Becton Dickinson). Positive cultures were subcultured on solid media and incubated at 37°C for 18 hours. Bacterial identification/speciation was done by biochemical testing (analytical profile index, triple sugar iron, lysine iron agar, and sulfur indole motility) and Gram staining. The antibiotic sensitivity testing was done by Kirby-Bauer disc diffusion method using Oxoid diffusion discs (Thermo Fisher, Waltham, MA), in accordance with Clinical and Laboratory Standards Institute guidelines. On selected isolates, multidrug resistance via ESBL production was confirmed using the Epsilometer (Etest) (AB Biodisk, Solna, Sweden) together with disc diffusion testing using clavulanic acid.

### Statistical Analysis

For comparisons of descriptive data between the study group and the broader admitted neonatal population and by maternal HIV status, categorical and continuous descriptive variables were compared by $\chi^2$ and Mann-Whitney *U* tests, respectively. Binary logistic regression was used to perform univariate and multivariate analysis of how a range of variables might affect the odds of culture-confirmed neonatal sepsis. Data analysis was done in SPSS version 21 (IBM, Armonk, NY).

## RESULTS

### Patient Recruitment and Descriptives

We approached the mothers/guardians of 342 admitted neonates with suspected neonatal sepsis during the study period. Three hundred twenty-one mothers/guardians consented to take part in the study. We failed to collect recruitment data before discharge/death of 8 participants, resulting in a final sample of 313 (Fig. 1); 54% (170/313) of the neonates were male and 20% (62/313) were born to HIV-infected mothers (Table 1); 79% (246/313) of neonates were born at the hospital, 13% (41/313) were referred from local clinics, 7% (21/313) from district hospitals and 2% (5/313) were home births; 79% (246/313) of neonates were admitted on the day they were born, 62% (194/313) were aged ≤3 days, 27% (84/313) were aged 4–7 days and 11% (35/313) were aged >7 days (Table 2). Compared with the total neonatal inpatient population during the study period, sex, maternal HIV exposure and mortality were broadly representative, but participants had a higher mean birth weight (Table 1).

Within the study sample, we then compared descriptive data between HIV-exposed and HIV-unexposed neonates. HIV-exposed neonates were significantly older: 3 [interquartile range (IQR), 2–5] vs. 2 (IQR, 1–5) days, $P = 0.029$, but had a lower mean birth weight: 2.1 kg (IQR, 1.6–2.8) vs. 2.7 kg (IQR, 1.8–3.2), $P = 0.003$ (Table 2). With respect to clinical presentation, HIV-exposed neonates were significantly more likely to present with cyanosis, distension, hepatomegaly or splenomegaly. The median age of HIV-infected mothers was 3 years older than HIV-uninfected mothers (27 years (IQR, 23–30) vs. 24 years (IQR, 19–29.5), $P = 0.008$, and correspondingly, median parity was also significantly higher for HIV-infected mothers (Table 2).

### Prevalence and Etiology of Neonatal Sepsis

Bacteria were cultured from the blood of 36% (113/313) of neonates. Probable contaminants accounted for 10 cases (6 diphtheroids, 3 mixed growth and 1 *Clostridium*), and so culture-confirmed sepsis was diagnosed in 33% (103/313) of neonates (Table 3). *Klebsiella* species, predominantly *K. pneumoniae*, were highly prevalent accounting for 75% (77/103) of all cases. Among the remaining 26 cases, *S. aureus* (6% (6/103), coagulase-negative staphylococci (6% (7/103), *E. coli (*5% (5/103) and *Candida* spp. (5% (5/103) were the most prevalent (Table 3). The prevalence of culture-positive sepsis was significantly higher among neonates aged 4–7 days [54% (45/84), $P = 0.001$] or >7 days [43% (15/35), $P = 0.006$] compared with those aged ≤3 days [22% (43/194); Table 3]. EOS accounted for 85% (88/103) of cases (Table 3).

### Antibiotic Resistance

Phenotypic drug-susceptibility testing was undertaken using a panel of antibiotics that are used locally, demonstrated near universal resistance (range 92%–100% resistant) to penicillin/ampicillin, gentamicin, trimethoprim-sulfamethoxazole, erythromycin and cephalosporins for Gram-negative rods (*Klebsiella* species and *E. coli*; Table 4). All but one *Klebsiella* isolate and all *E. coli* isolates were resistant to multiple first- and second-line antibiotics. For *Klebsiella* spp., antibiotic resistance ranged from 96–99% for WHO-recommended first-line therapy (gentamicin and ampicillin/

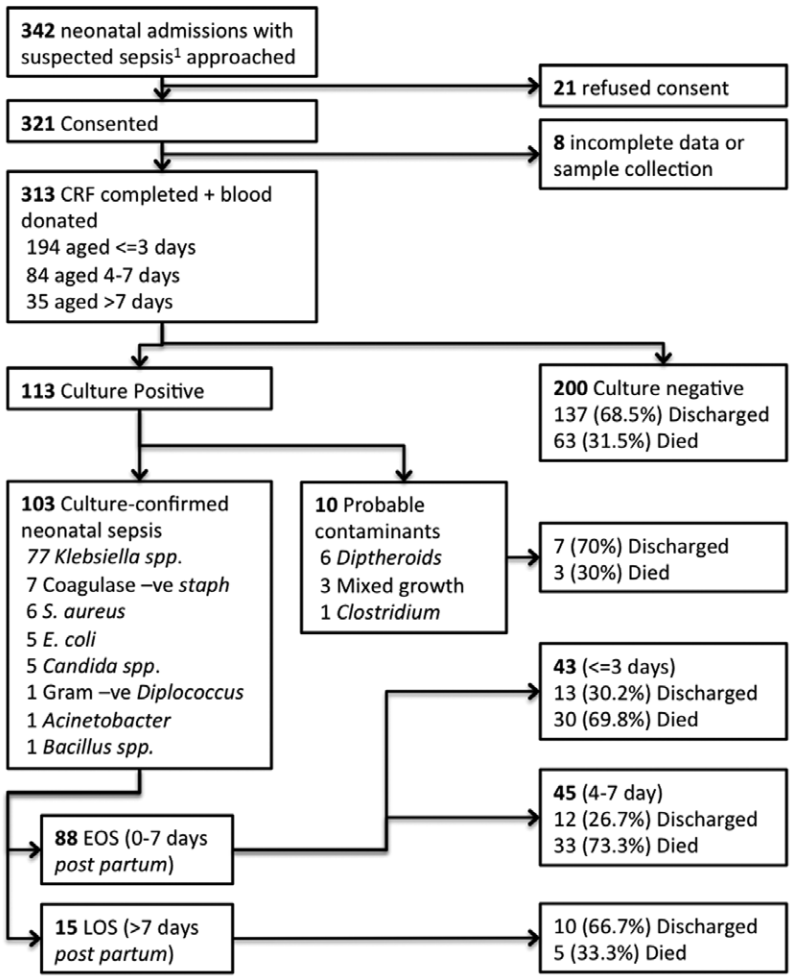


**FIGURE 1.** Study flow diagram. CRF indicates Clinical Recruitment Form.

penicillin) to 94–97% for third-generation cephalosporins. Phenotypic analysis of selected *Klebsiella* and *E. coli* isolates indicated the presence of ESBLs (data not shown). We identified only one *K. pneumoniae* isolate that was also resistant to third-line therapy (imipenem).

### *Klebsiella* Outbreak

During the study period, we documented an apparent outbreak of *Klebsiella* infections among neonates admitted with suspected sepsis, with prevalence increasing from 0% in December 2013 to 39% in March 2014 (Fig. 2). During this period, the mortality rate among study participants increased from 29% to 47%, with an increase in mortality also documented within all NICU admissions during the same period (Fig. 2); 93% (14/15) of LOS cases had been admitted for >2 days before the onset of symptoms (Fig. 3). For EOS cases aged 4–7 days, 82% (37/45) of cases had been admitted for >2 days. For culture-confirmed EOS in neonates aged ≤3 days, the distribution by days to onset of symptoms appears bimodal, possibly delineating those where the infection was contracted on the birthing wards or theater, from those who contracted the infection upon arrival to the neonatal unit (Fig. 3).

### Impact of Culture Diagnosis on Mortality

The overall mortality rate was 43% (134/311; Table 3). For neonates aged ≤7 days, the mortality rate among neonates with a positive culture was 72% (63/88) compared with 30% (57/190) among culture-negative neonates ($P < 0.001$). For neonates aged >7 days, the mortality rate did not differ significantly between

**TABLE 1.** Comparison of Key Descriptive Variables Between the Study Group and Admitted Neonatal Population From Which the Study Group Was Recruited

| | Study (n = 313) | Population (n = 2471) | Significance |
|---|---|---|---|
| Male sex (%) | 54.3% (170/313) | 50.3% (1248/2469) | 0.209 |
| Mean weight in kg (SE) | 2.5 (0.048) | 2.2 (0.02) | <0.001 |
| Maternal HIV infection (%) | 19.7% (60/305) | 17.3% (413/2389) | 0.303 |
| Mortality (%) | 43.2% (134/310) | 43.2% (1067/2470) | 1.000 |

SE indicates standard error.

**TABLE 2.** Demographic and Clinical Characteristics of Neonates and Mothers Stratified by Maternal HIV Status

| | All Neonates | Neonates Born to HIV-uninfected Mothers | Neonates Born to HIV-infected Mothers | *P** |
|---|---|---|---|---|
| Neonates | | | | |
| Male sex | 54.3% (170/313) | 54.3% (133/245) | 58.3% (35/60) | 0.572 |
| Median age in days (IQR) | 2 (1–5) | 2 (1–5) | 3 (2–5) | 0.029 |
| Aged ≤3 d | 62% (194/313) | 83% (161/194) | 17% (33/194) | 0.276 |
| Aged 4–7 d | 27% (84/313) | 76% (64/84) | 24% (20/84) | |
| Aged >7 d | 11% (35/313) | 74% (26/35) | 26% (9/35) | |
| Mean weight in kg (SE) | 2.6 (2.2) | 2.6 (0.05) | 2.2 (0.1) | 0.003 |
| Hospital birth | 78.6% (246/313) | 78% (191/245) | 80% (48/60) | 0.731 |
| Delivery type | | | | |
| Spontaneous vaginal delivery | 78.6% (246/313) | 76.7% (188/245) | 85.0% (51/60) | 0.205 |
| Cesarean section | 18.2% (57/313) | 19.6% (48/245) | 15.0% (9/60) | |
| Instrument | 3.2% (10/313) | 3.7% (9/245) | 0.0% (0/60) | |
| Clinical presentation† | | | | |
| Fever | 62.3% (195/313) | 62% (152/245) | 61.7% (37/60) | 0.957 |
| Cyanosis | 13.1% (41/312) | 11.5% (28/245) | 21.7% (13/60) | 0.038 |
| Distension | 10.2% (32/313) | 8.6% (21/245) | 18.3% (11/60) | 0.027 |
| Hepatomegaly | 5.4% (17/313) | 4.1% (10/245) | 11.7% (7/60) | 0.022 |
| Splenomegaly | 3.8% (12/313) | 2% (5/245) | 11.7% (7/60) | 0.001 |
| Mortality | 43.2% (134/310) | 43% (104/242) | 41.7% (25/60) | 0.854 |
| Mothers | | | | |
| Median age in years (IQR) | 25 (20–29.5) | 24 (19–29.5) | 27 (23–30) | 0.008 |
| Mother's education‡ | | | | |
| None | 6.2% (17/273) | 6% (12/211) | 7% (4/54) | 0.750 |
| Primary | 32.2% (88/273) | 32% (67/211) | 37% (20/54) | |
| Secondary | 54.6% (149/273) | 56% (118/211) | 48% (26/54) | |
| Tertiary | 7% (19/273) | 7% (14/211) | 7% (4/54) | |
| Married | 83.3% (254/305) | 82% (196/239) | 88% (52/59) | 0.259 |
| Parity | 2 (1–3) | 2 (1–3) | 3 (1.3–4) | 0.012 |
| Complications§ | 44.7% (140/313) | 47.3% (116/245) | 38.3% (23/60) | 0.209 |

PV indicates per vaginal; RBS, random blood sugar; WCC, white cell count.

*Univariate analysis by $\chi^2$ test for categorical variables, and Mann-Whitney *U* for continuous variables, none of which were determined to have a normal distribution.

†The prevalence of the following did not differ by maternal HIV status: hypothermia 8.0% (25/313); convulsions 30.4% (95/313); poor feeding 47.3% (148/313); vomiting 1.9% (6/313); difficulty breathing 62.3% (195/313); tachypnea 71.9% (225/313); chest recession 31.0% (97/313); nasal flaring 31.0% (97/313); pallor 11.2% (35/313); jaundice 11.5% (36/313); irritability 42.5% (133/313); lethargy 26.2% (82/313); bulging fontanel 7.3% (23/313); umbilical discharge 2.6% (8/313); eye infection 4.5% (14/313). Also vital signs: temperature (°C)—38.0 (36.4–38.5); respiratory rate (breaths/min)—52 (48–60); pulse (pulses/min)—150 (140–160) and hematology: WCC (cells/mL)—15.4 (12.0–19.6); Hb (g/L)—14.5 (13.4–16.1); platelet (1000 plates/mL)—152 (110–210) and RBS (g/L)—4.3 (3.2–6.1).

‡Maternal education was collected for 273 cases.

§The prevalence of the following complications in pregnancy did not differ by maternal HIV status: abdominal pain [29.4% (92/313)]; pain when passing urine [8.9% (28/313)]; pain when having sexual intercourse [2.2% (7/313)]; PV spotting [12.8% (40/313)]; vaginal discharge [3.5% (11/313)]; rash [1% (3/313)]; fever [4.8% (15/313)].

**TABLE 3.** Predominant Pathogens Isolated

| | | | Prevalence Within Different Age Groups | | |
|---|---|---|---|---|---|
| | Count | Overall Prevalence | Aged ≤3 days (194) | Aged 4–7 days (84) | Aged <7 days (35) |
| Any positive culture | 103 | 33% (103/313) | 22% (43/194)†‡ | 54% (45/84)† | 43% (15/35)‡ |
| Established pathogens | | | | | |
| *Klebsiella* species* | 77 | 25% (77/313) | 13% (25/194)†§ | 49% (41/84)† | 31% (11/35)§ |
| *S. aureus* | 6 | 2% (6/313) | 2% (4/194) | 1% (1/84) | 3% (1/35) |
| *E. coli* | 5 | 2% (5/313) | 2% (3/194) | 2% (2/84) | 0% (0/35) |
| Probable pathogens in the African setting | | | | | |
| Coagulase-negative *staphylococci* | 7 | 2% (7/313) | 3% (6/194) | 0% (0/84) | 3% (1/35) |
| *Candida* species | 5 | 2% (6/313) | 2% (4/194) | 1% (/84) | 0% (0/35) |
| Gram-negative diplococcus | 1 | <1% (1/313) | 0% (0/194) | 0% (0/84) | 3% (1/35) |
| *Bacillus* species | 1 | <1% (1/313) | 0% (0/194) | 0% (0/84) | 3% (1/35) |
| *Acinetobacter* | 1 | <1% (1/313) | 0.5% (1/194) | 0% (0/84) | 0% (0/35) |

**Klebsiella pneumoniae* (76) and *Klebsiella oxytoca* (1).

†Pearson $\chi^2$ $P < 0.001$.

‡Pearson $\chi^2$ $P = 0.006$.

§Pearson $\chi^2$ $P = 0.009$.

**TABLE 4.** Antibiotic Resistance

| | | *Klebsiella* species* | *Staphylococcus aureus* | *Escherichia coli* |
|---|---|---|---|---|
| Prevalence of pathogen | | 25% (77/313) | 1.9% (6/313) | 1.6% (5/313) |
| Prevalence of antibiotic resistance | | | | |
| Antibiotic class | Antibiotic | | | |
| Penicillins | Penicillin/ampicillin | 99% (68/69) | 33% (1/3) | 100% (5/5) |
| | Amoxicillin/clavulanic acid | 93% (50/54) | 100% (4/4) | 50% (1/2) |
| | Oxacillin | NT | 0% (0/3) | NT |
| Aminoglycoside | Gentamicin | 96% (70/73) | 50% (2/4) | 100% (5/5) |
| Sulfonamide | Trimethoprim-sulfamethoxazole | 100% (54/54) | 67% (2/3) | 100% (5/5) |
| Macrolide | Erythromycin | 92% (49/53) | 33% (1/3) | 100% (5/5) |
| Amphenicol | Chloramphenicol | 71% (52/73) | 20% (1/5) | 60% (3/5) |
| Quinalone | Ciprofloxacin | 71% (51/72)† | 0% (0/5) | 80% (4/5) |
| Cephalosporins | Ceftriaxone | 94% (48/51) | 33% (1/3) | 100% (5/5) |
| | Cefotaxime | 96% (71/74) | 0% (0/5) | 100% (5/5) |
| | Ceftazidime | 97% (64/66) | 50% (1/2) | 100% (5/5) |
| Carbapenem | Imipenem | 1% (1/73) | 0% (0/3) | 0% (0/5) |

NT indicates not tested.
**Klebsiella pneumoniae* (76) and *Klebsiella oxytoca* (1).
†For ciprofloxacin testing, there were 10 intermediate results.

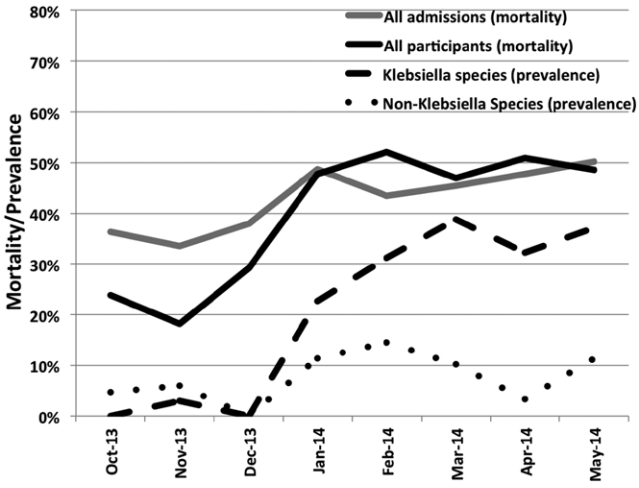


**FIGURE 2.** Prevalence of *Klebsiella* species and non-*Klebsiella* species, and mortality among study participants and overall within the neonatal unit, by month.

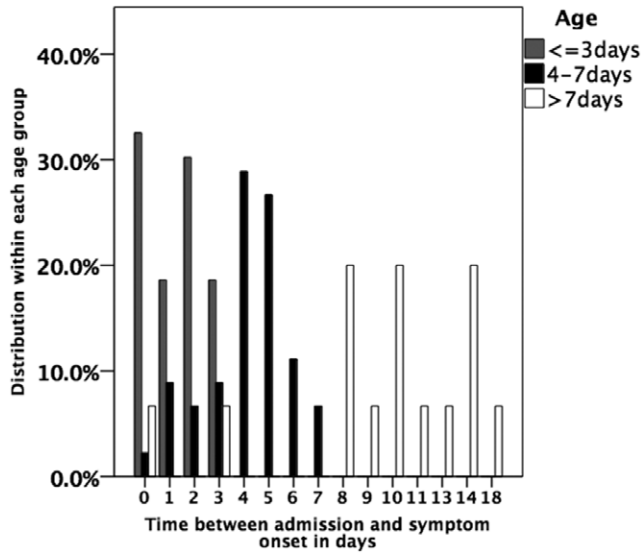


**FIGURE 3.** Days from admission to onset of symptoms for culture-confirmed EOS and LOS cases.

culture-positive [33% (5/15)] and culture-negative [45% (9/20)] cases ($P = 0.486$; data not shown). In accordance with routine practice at the hospital, both species and drug-susceptibility testing data are reported together. The median reporting time on the study was 7 days (IQR, 5–9 days), but the median times to discharge or death were 6 (IQR, 3–8) and 3 (IQR, 1–6) days, respectively (data not shown). Only 25% (26/103) of culture-confirmed cases of neonatal sepsis received a culture result before discharge or death (data not shown).

## Risk Factors Associated With Neonatal Sepsis

The odds of culture-positive neonatal sepsis were significantly reduced in children born to HIV-infected mothers {OR, 0.46 [95% confidence interval (CI), 0.23–0.93], $P = 0.029$}, independent of age, parity, hepatomegaly, irritability and poor feeding, which were flagged by univariate analysis (Table 5). This finding also held when adding birth weight and age into the model [OR, 0.42 (95% CI, 0.21–0.86), $P = 0.018$; data not shown]. Restricting this analysis to neonates ≤3 days of age, this lost significance [OR, 0.57 [95% CI, 0.19–1.7], $P = 0.301$], but among neonates aged 4–7 days [OR, 0.29 (95% CI, 0.09–0.99), $P = 0.048$] and those aged >7 days [OR, 0.09 (95% CI, 0.01–0.83), $P = 0.034$], the negative association between HIV-exposed and HIV-positive culture was maintained (data not shown). Increasing neonatal age and increasing parity were independently associated with increased odds of culture-positive sepsis (Table 5). Conversely, nasal flaring and pallor were independently associated with reduced odds of neonatal sepsis (Table 5). Analyzing HIV-exposed and HIV-unexposed children separately did not reveal any additional risk factors. Odds of neonatal sepsis did not differ significantly between neonates born at the referral center and those born in the community (Table 5).

## DISCUSSION

There were 4 key findings from this study: (a) We documented a high prevalence of neonatal sepsis associated with an apparent nosocomial outbreak of multidrug-resistant *K. pneumoniae*; (b) for *K. pneumoniae*, resistance to WHO-recommended first- and second-line antibiotics was almost universal; (c) results

**TABLE 5.** Risk Factors for Culture-positive Neonatal Sepsis

| | Odds of Positive Blood Culture | | | |
|---|---|---|---|---|
| | Univariate | | Multivariate | |
| | OR (95% CI) | *P* | OR (95% CI)* | *P* |
| Neonates | | | | |
| Male sex | 1.00 (0.63–1.61) | 0.989 | 0.97 (0.59–1.59) | 0.965 |
| Infant age (d) | 1.06 (1.01–1.11) | 0.031 | 1.07 (1.01–1.13) | 0.021 |
| Birth weight | 0.83 (0.63–1.10) | 0.201 | 0.87 (0.64–1.18) | 0.360 |
| Born in referral center | 0.61 (0.35–1.06) | 0.082 | 0.69 (0.38–1.24) | 0.214 |
| Delivery type† | | | | |
| Cesarean section | 0.56 (0.29–1.10) | 0.091 | 0.49 (0.23–1.01) | 0.054 |
| Instrument | 1.89 (0.53–6.73) | 0.323 | 2.01 (0.49–8.29) | 0.332 |
| Clinical presentation‡ | | | | |
| Poor feeding | 0.60 (0.37–0.97) | 0.036 | 0.68 (0.41–1.13) | 0.132 |
| Nasal flaring | 0.65 (0.38–1.10) | 0.106 | 0.54 (0.31–0.96) | 0.034 |
| Pallor | 0.47 (0.2–1.13) | 0.090 | 0.36 (0.14–0.94) | 0.037 |
| Irritability | 1.66 (1.03–2.67) | 0.038 | 1.36 (0.82–2.26) | 0.228 |
| Hepatomegaly | 0.12 (0.02–0.91) | 0.040 | 0.17 (0.02–1.39) | 0.098 |
| Mothers§ | | | | |
| Increasing parity | 1.18 (1.02–1.37) | 0.023 | 1.18 (1.01–1.37) | 0.032 |
| Maternal HIV infection¶ | | | | |
| HIV infected | 0.49 (0.25–0.96) | 0.038 | 0.46 (0.23–0.93) | 0.029 |
| HIV status unknown | 0.60 (0.12–3.01) | 0.530 | 0.20 (0.02–1.97) | 0.158 |

*Multivariate analysis controlling for age, poor feeding, irritability, hepatomegaly, parity and maternal HIV status indicated by univariate analysis.

†Compared with spontaneous vaginal delivery as reference category.

‡No significant associations were observed for auxiliary temperature, fever, hypothermia, vomiting, difficulty breathing, tachypnoea, chest recessions, jaundice, cyanosis, lethargy, distension, umbilical discharge, eye infection, bulging fontanel, convulsions and splenomegaly.

§No significant associations were observed for age, fever, abdominal pain, urination pain, vaginal discharge, vaginal bleeding or pain during sex.

¶Compared with HIV-uninfected as reference category.

of blood cultures and speciation were available before discharge or death in only 25% of cases; (d) in multivariate analysis, maternal HIV infection was associated with a 2-fold reduction in the odds of neonatal sepsis.

The study had several limitations. Interpretation of mortality data was confounded by the cross-sectional study design, which did not collect serial blood cultures, with the possibility of missed neonatal sepsis-associated mortalities. As such, we do not present a comprehensive multivariate analysis on the impact of sepsis on mortality. However, among mortalities that were culture negative, the median number of days from recruitment to mortality was just 1 day (IQR, 0–4 days; data not shown), suggesting that the window of opportunity for missed culture-positive sepsis-associated mortalities was quite small. The study was undertaken at a single site, and so the findings may not be representative of other neonatal units. There was selection bias against babies with lower birth weight, who were underrepresented in the sample. Obtaining specimens for analysis and maternal consent are both more challenging for premature neonates. Gestational age was not recorded, preventing more detailed analysis comparing small for gestational age with acceptable gestational age neonates.

The overall prevalence of culture-positive sepsis was 33% (103/313), a possible underestimate as we did not perform serial blood cultures. This is 50% higher than the pooled prevalence of 22% from 14 previous African studies.[4,6–8,11–15,27–31] *K. pneumoniae* accounted for 75% (77/103) of all positive cultures and correlated with an associated increase in mortality, both within the study population and within all neonatal admissions during the study period. The study was limited, in that we did not screen mothers, but the dramatic increase in infections from December 2013 to March 2014 correlating with mortality, the timing of infection after admission, the identity of the pathogen (*K. pneumoniae*) and the detection of *K. pneumoniae* from surface swabs on both labor ward, theater and the neonatal unit (data not shown), are strongly indicative of a nosocomial outbreak. Persistently high mortality rates on the unit before and after the study period (data not shown) suggest that such outbreaks may be the *status quo*, interspersed with short periods of lower prevalence, likely influenced by multiple factors, including enhanced infection control activities and staff rotations. Efforts to promote safe birthing practices and increase the proportion of institutional deliveries may be inadvertently putting neonates at risk, if corresponding efforts are not also focused on developing birthing center capacity and ensuring rigorous infection control.[32]

For *K. pneumoniae*, we observed near universal resistance to both first-line (gentamicin and ampicillin/penicillin) and second-line therapy (third-generation cephalosporins) driven by ESBLs. This level of resistance is higher than in earlier studies,[17,20,22] but consistent with more recent reports, which suggest ESBLs are an "ever-growing burden" in both hospital and community settings, affecting patients across all age groups.[33] WHO-recommended first- and second-line antibiotic therapy are impotent in the face of outbreaks of ESBL *Enterobacteriaceae*, and so the focus must be on improved infection control. We identified just one isolate that was resistant to imipenem, and so there is a window of opportunity to reduce the burden of neonatal infections, before levels of carbapenem resistance[24] mirror what we are now seeing with ESBLs.

Simple and scalable infection control interventions can reduce the burden of multidrug-resistant bloodstream infections in neonates,[9] but there is also a need for improved diagnostic tools and strategies. Although bacterial culture is a valuable research tool, it is expensive, slow and here offered little tangible benefit for high mortality risk neonates, with results available before discharge or death for only 25% (26/103) of cases. There is a need for novel rapid diagnostic tests, using DNA or other biomarkers,

which can quickly provide clinically relevant life-saving information on pathogen species and antibiotic susceptibility. However, the mortality rate among culture-negative neonates on our study was also very high (31%), similar to mortality rates among culture-positive cases in previously documented ESBL-*Klebsiella* outbreaks (30–36%),[34,35] suggesting that at our center, while infection is clearly important, efforts to control, diagnose and treat infections in neonates need to be synergized with improved obstetric and neonatal nursing care.

The low burden of Gram-positive infections and absence of GBS suggests that, in the hospital setting, these infections are either less common or are outcompeted by drug-resistant Gram-negative pathogens, consistent with the lower levels of drug resistance seen for the limited number of *S. aureus* isolates. GBS are a common cause of neonatal sepsis and meningitis in high-income regions where they are an established priority for vaccine development.[36] The absence of GBS on this study, and low prevalence on other African studies,[7,8,10,30,37] raises questions over the possible impact of a GBS vaccine in the African setting.[38]

There has been huge progress over the last decade in reducing MTCT of HIV, initially through single-dose nevirapine, but increasingly through more extensive antiretroviral regimens.[39] The dramatic reduction in the rate of MTCT has led to a growing number of infants who are HIV-exposed but uninfected.[40] This group, despite remaining HIV-uninfected, are known to suffer from increased morbidity and mortality, suffer from impaired growth and development[40,41] and are at greater risk of sepsis and other infections.[42] In our study, HIV-exposed neonates were at decreased risk of neonatal sepsis. This sizeable effect (a halving of the odds) was counterintuitive, but a South African study also found rates of neonatal sepsis to be marginally lower in HIV-exposed children.[43] The finding held when controlling for other covariates and neonatal antibiotic treatment at recruitment did not differ by maternal HIV status (data not shown). The most obvious therapeutic difference between HIV-exposed and HIV-unexposed neonates is the use of nevirapine prophylaxis in HIV-exposed neonates and probable maternal trimethoprim-sulfamethoxazole prophylaxis. A previous study looking at the incidence of gastrointestinal and respiratory infections over time demonstrated reduced mortality in the nevirapine arm, in both HIV-infected and HIV-exposed neonates.[44] In vitro testing of nevirapine against a panel of commercial strains and clinical isolates did not identify any antimicrobial activity, although *Klebsiella* species were not included in this study.[45] Reverse transcriptase enzymes have been identified in *E. coli*,[46] and azidothymidine has reported antimicrobial activity in a range of Gram-negative bacteria including *K. pneumoniae*.[47] Both these studies date back to the 1980s, and the authors of this article could not find any more recent data. A limitation of the study was that we did not collect data on maternal antibiotic history, but Zambia follows WHO guidelines which recommend trimethoprim-sulfamethoxazole prophylaxis in all HIV-infected women throughout pregnancy and while breastfeeding, and trimethoprim-sulfamethoxazole is also detectable in breast milk.[48] As all but one of the bacterial pathogens tested on this study were resistant to trimethoprim-sulfamethoxazole, it is maybe the reported immunomodulatory effects of the drug[48] that might be offering some level of protection from neonatal sepsis.

In summary, our study has shown that *K. pneumoniae* is an important cause of neonatal sepsis at our UTH, associated with high levels of mortality, and that WHO-recommended first- and second-line therapy are redundant in the face of rampant multidrug resistance and inadequate infection control. In this setting, blood culture has minimal clinical impact, and there is a need for improved infection control and novel molecular diagnostics for bacterial bloodstream infections (on par with those developed for HIV and malaria) that can rapidly inform on life-saving treatment interventions in high mortality risk neonates. The finding that a factor linked to HIV-exposed neonates is associated with reduced risk of neonatal sepsis requires further detailed investigation in vitro and in vivo.

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