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Original Article

Epidemiology of clinically suspected and laboratory-confirmed bloodstream infections at a South African neonatal unit

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Abstract

Introduction: Data from Africa reporting the epidemiology of infection in hospitalised neonates are limited.

Methodology: A prospective study with convenience sampling was conducted to characterise neonates investigated with blood culture/s for suspected infection at a 132-bed neonatal unit in Cape Town, South Africa (1 February-31 October 2018). Enrolled neonates were classified as having proven bloodstream infection (BSI) (blood culture-positive with a pathogen) or presumed infection (clinically suspected but blood culture-negative) or as potentially at risk of infection (maternal risk factors at birth).

Results: Of 1299 hospitalised neonates with ≥1 blood culture sampling episode, 712 (55%) were enrolled: 126 (17.7%) had proven BSI; 299 (42%) had presumed infection and 287 (40.3%) were potentially at risk of infection. Neonates with proven BSI had lower birth weight and higher rates of co-existing surgical conditions versus the presumed/potential infection groups ($p < 0.001$). Median onset of proven BSI versus presumed infection was at 8 (IQR = 5-13) and 1 (IQR = 0-5) days respectively ($p < 0.001$). Most proven BSI were healthcare-associated (114/126; 90.5%), with *Klebsiella pneumoniae* (80.6% extended-spectrum β-lactamase producers) and *Staphylococcus aureus* (66.7% methicillin-resistant) predominating. Mortality from proven BSI (34/126; 27%) was substantially higher than that observed in presumed (8/299; 2.7%) and potential infections (3/287; 1.0%) ($p < 0.001$). The odds of death from proven BSI was 3-fold higher for Gram-negatives than for Gram-positive/fungal pathogens (OR = 3.23; 95% CI = 1.17-8.92).

Conclusions: Proven BSI episodes were predominantly healthcare-associated and associated with a high case fatality rate. Most neonates with presumed infection or at potential risk of infection had favourable 30-day outcomes.

Key words: neonate; infant; bloodstream infection; bacteraemia; sepsis; antimicrobial resistance; Africa.

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Introduction

Severe bacterial infections, including bloodstream infection (BSI) cause an estimated 750 000 neonatal deaths in low-middle income countries (LMIC) annually [1]. Ironically, settings with the highest neonatal infection burden seldom report BSI surveillance data. Two systematic reviews identified no studies of national neonatal infection incidence identified from Sub-Saharan Africa [2,3]. Limited access to microbiology laboratories, low blood culture yields and requirement for out-of-pocket payment for diagnostic tests, are some of the barriers to diagnosis of bacterial infection in African neonatal units [4]. The lack of validated clinical neonatal infection definitions also hampers efforts to measure infection burden.

Given these diagnostic challenges for neonatal infections in LMIC, categories have been developed utilising clinical judgement to ascribe the degree of infection certainty [5]. Proven infections include neonates with isolation of a known neonatal pathogen on blood culture or other sterile site e.g. meningitis and urinary tract infection. Presumed infections (also referred to as clinically-suspected of culture-negative infection episodes, occur in neonates with symptoms and signs of infection at birth or during their hospital stay but no identifiable pathogens despite comprehensive laboratory testing. Lastly, some neonates are at risk for potential infection owing to maternal factors such as chorioamnionitis and prolonged rupture of membranes.

The risk for neonatal HA-BSI in African hospitals is disproportionately high and influenced by high prematurity and low birth weight rates that necessitate longer hospital stays. Increasing in-hospital births in

Africa, healthcare facility overcrowding and chronic understaffing of maternity and newborn services are also important drivers of neonatal infection risk [6,7]. In South Africa, infections account for at least 14% of neonatal deaths, with hospital-acquired infection (HAI) being the most frequent avoidable cause of in-hospital neonatal mortality [8]. The rate of multidrug-resistant (MDR) Gram-negative pathogen acquisition in hospitalised neonates is substantial, with pathogens either vertically-acquired from colonised mothers [9] or horizontally-transferred from the hospital environment through shared equipment, inadequate cleaning and poor infection-prevention practices [10-12]. *Klebsiella pneumoniae* is the leading neonatal pathogen implicated in half of neonatal unit outbreaks [13] and associated with mortality rates ranging from 16% to 100% [14]. In some African neonatal units, carbapenem-resistant *Acinetobacter baumannii* and Enterobacterales (CRE) are now leading causes of neonatal infectious deaths, especially in preterm infants [15-18]. In this paper we characterise the epidemiology of clinically suspected and laboratory-confirmed bloodstream infections at a large academic neonatal unit in South Africa, with analysis of factors associated with mortality in proven infections.

Methodology

Study design and population

A prospective study of neonates evaluated for suspected bacterial infection was conducted at the Tygerberg Hospital Neonatal Unit, Cape Town, South Africa between 1 February-31 October 2018. Any baby hospitalised on a neonatal ward or in the neonatal intensive care unit (NICU) with 1 or more blood cultures submitted during the study period was eligible for enrolment. The enrolment strategy entailed convenience sampling of eligible neonates on weekdays only, where the baby's mother was on-site and willing to provide written informed consent to data collection and 30-day follow-up. The Stellenbosch University Health Research Ethics Committee and the Tygerberg Hospital management reviewed and approved the study protocol (N18/07/068).

Study setting

Tygerberg Hospital is a 1,384-bed public teaching hospital. Despite being classified as an upper middle-income country by the World Bank, South Africa has one of the world's highest Gini coefficients indicating inequality [19]. Most patients utilizing the public healthcare service are indigent and more typical of the population from LMIC. In 2017, the antenatal HIV prevalence in the Western Cape Province was 15.9% (95% CI = 14.2%–17.8%), with universal antiretroviral therapy in pregnancy and a national mother-to-child HIV infection transmission rate of 0.9% [20]. Tygerberg Hospital's busy obstetric-neonatal service manages approximately 8000 high-risk deliveries (37% low birth weight rate) and 3,000 neonatal admissions annually.

Description of the neonatal unit

The 132-bed neonatal unit includes a 12-bed NICU, three high-dependency wards, and one kangaroo mother care ward, with mean occupancy rates exceeding 100%. The neonatal unit provides medical and surgical care for sick, preterm (< 37 weeks' gestation) and/or low-birthweight (< 2,500 g) inborn and outborn neonates from surrounding district hospitals and midwife obstetric units. Prematurity, perinatal asphyxia and infection are the most common indications for admission. Critically ill neonates are nursed in the NICU, with availability of respiratory support (conventional ventilation, oscillation and nasal continuous positive airway pressure [nCPAP]), inotropic support and nitric oxide therapy. Given the extreme shortage of NICU beds, non-invasive ventilation (nCPAP and high-flow oxygen therapy) is used extensively on the high-dependency wards. Central lines (umbilical venous catheters and peripherally inserted central catheters) are used in all wards; a central line-associated BSI (CLABSI) prevention programme was launched in the NICU in 2012, and subsequently expanded to 2 other neonatal wards. The hospital's on-site Unit for Infection Prevention and Control (IPC), has one infection prevention nurse practitioner dedicated to the maternity, paediatric and neonatal departments. The Unit for IPC disseminates monthly surveillance reports on BSI and blood culture contamination rates in the neonatal unit.

Clinical evaluation of suspected neonatal infection

Indications for neonatal infection evaluation at Tygerberg Hospital include: maternal risk factors for infection at birth (e.g. chorioamnionitis, prolonged rupture of membranes, unexplained preterm delivery); and neonatal signs of infection at birth or during hospital stay. These include abnormal vital signs (temperature, glucose, respiratory and/or heart rate), and signs or symptoms suggestive of infection (respiratory distress, apnoea, lethargy, poor feeding, abdominal distention, vomiting). For babies with maternal risk factors at birth, a blood culture and full

blood count is collected, with delayed collection of blood for C-reactive protein (CRP) testing at 24-48 hours, with antibiotic discontinuation for well babies with CRP < 10mg/dL and where blood culture has not flagged positive. For babies with suspected infection at birth or during hospitalisation, a full blood count, CRP and a single blood culture sample is obtained by peripheral blood draw. CRP is widely used in this unit as an antibiotic stewardship tool to reduce neonatal antibiotic duration, when possible. Additional blood cultures may be obtained through a central line for infants with suspected CLABSI.

Antimicrobial management of neonatal infection

The hospital's guideline for empiric antibiotic therapy for suspected neonatal infection recommends ampicillin plus gentamicin for early-onset infection (< 72 hours of life). For HAI (≥ 72 hours of life) piperacillin-tazobactam plus amikacin are empirically prescribed for stable neonates, and meropenem for critically ill neonates or neonates with suspected meningitis. For patients with HAI in the presence of thrombophlebitis or recent use of central lines, vancomycin is added at the clinician's discretion. Antifungal therapy (amphotericin B or fluconazole) is added for selected neonates with risk factors for invasive fungal infection (e.g. abdominal surgery, persistent thrombocytopaenia) at the discretion of the neonatal consultant; the unit does not use routine fluconazole prophylaxis.

Laboratory procedures for blood culture processing

The onsite National Health Laboratory Service uses the automated BacT/Alert blood culture system (BioMerieux, Marcy l'Etoile, France). Local guidelines recommend inoculating at least 1–2 mL of blood into a paediatric blood culture bottle (BacT/ALERT PF bottle). If bacterial growth is detected, a Gram stain is performed and the sample sub-cultured onto appropriate media and incubated overnight. Further identification and antimicrobial susceptibility testing of clinically significant isolates is performed with the automated Vitek II system (BioMerieux) using Clinical and Laboratory Standards Institute (CLSI) breakpoints [21]. If urinary tract infection, meningitis or another infection focus is suspected, additional laboratory specimens are submitted.

Data definitions and sources

Patient records and hospital admissions data were utilised to collect data on patient demographics, clinical and antimicrobial management of infection episodes, length of stay and 30-day outcome. Laboratory records were used to collect data on blood investigations, blood culture pathogen identification and antimicrobial susceptibility patterns. Clinical outcome at 30-days after blood culture collection was confirmed by folder review for neonates still hospitalised and telephonically for those that had been discharged or transferred. Caregivers and their neonates who could not be telephonically traced following discharge or transfer were indicated as "lost to follow-up" at the 30-day outcome check.

The following standard definitions were used to stratify neonates: low birthweight (< 2,500 g), very low birthweight (1,000-1,500 g) and extremely low birthweight (< 1,000 g). Inborn neonates refers to babies born at Tygerberg Hospital, whereas outborn refers to those born at another hospital, midwife obstetric unit or born before arrival. Prior antibiotic therapy was defined as administration of one/more systemic antibiotic doses (documented in the prescription chart) that occurred prior to the current infection episode. Definitions proposed by Wirtschafer [5] were used to classify infection episodes as: proven BSI (blood culture-proven sepsis); presumed infection (clinical sepsis, blood culture-negative) and potential infection (asymptomatic neonate at high risk for infection at birth).

A BSI episode was defined as a blood culture yielding a pathogen, including repeat cultures isolating the same pathogen within 10 days of the original specimen. HA-BSI were defined as a positive blood culture yielding a known neonatal pathogen (based upon the categorization of the United States Centers for Disease Control, US CDC) [22] obtained at ≥ 72 hours of life/hospitalization. Coagulase-negative staphylococci (CoNs) were classified as pathogens if the same species was isolated from a repeat blood culture from a separate blood draw collected on the same/subsequent day. If a contaminant was isolated, the blood culturing episode was allocated to the potential infection group (if they had infection risk factors only) or the presumed infection group (if they had clinical symptoms/signs of infection, irrespective of risk factors for infection).

BSI-attributable death was defined as occurring within 72 hours of blood collection that established a proven BSI, where the treating clinician considered the neonate's demise to be a consequence of the BSI and/or its infectious complications [23]. Data regarding the patient's demographic profile, infection episode laboratory results, pathogen and antimicrobial

resistance spectrum and 30-day outcome were entered into a REDCap database [24].

Antibiotic susceptibility patterns

The following susceptibility patterns were regarded as an antibiotic–resistant phenotype: methicillin resistance in *S. aureus*; third or fourth generation cephalosporin resistance in *E. coli* and *K. pneumonia*e (likely extended-spectrum β-lactamase production - ESBL); fourth generation cephalosporin resistance in *E. cloacae* and *S. marcescens* (ESBL or derepressed AmpC), carbapenem resistance in *A. baumannii* or *P. aeruginosa,* and azole resistance in *Candida* species.

Statistical analysis

Continuous and categorical variables were compared using the Kruskal-Wallis test and the X^2 test, respectively. To determine factors associated with mortality from LC-BSI, intelligent multivariable logistic regression analyses were performed. A *p*-value of < 0.05 was considered statistically significant. Stata Statistical Software version 13.0 IC (College Station, TX: StataCorp LP) was used for analysis.

Ethical approval

The Stellenbosch University Health Research Ethics Committee and the Tygerberg Hospital management reviewed and approved the study protocol (N18/07/068).

Results

Characteristics of the study population

During the study period, 1,299 neonates had 1 or more blood culture submitted. We enrolled 712/1,299 (55%) neonates: 126 (17.7%) had proven infection, 299 (42%) had presumed infection, and 287 (40.3%) were at potential risk of infection (Figure 1). Most enrolled neonates were preterm and/or of low birth weight and not HIV-exposed (Table 1). Among HIV-exposed neonates (136/712; 19.1%), mother-to-child transmission (MTCT) of HIV rates did not differ between groups (3.7% overall). Babies with proven BSI were more likely to be born by caesarean section, preterm, of low birth weight, previously treated with antibiotics and to have underlying surgical conditions (all $p < 0.001$).

Table 1. Demographic and clinical characteristics of the study population (N = 712).

Demographic variable		Proven bloodstream infection	Presumed infection	Potential infection	*p*-value
		blood culture-proven sepsis N = 126	clinical sepsis,blood culture-negative N = 299	asymptomatic neonate atrisk of infection at birth N = 287	
Male gender, n (%)		66 (52.4)	156 (52.2)	141 (49.1)	0.105
Gestational age in weeks, median (IQR)		29 (28-32)	31 (29-36)	32 (30-34)	< 0.001
Birth weight in grams, median (IQR)		1128 (890-1535)	1500 (1090-2370)	1625 (1240-2025)	< 0.001
Maternal HIV status, n (%)	HIV-negative	102 (81.0)	240 (80.3)	234 (81.5)	0.045
	Living with HIV on antiretroviral therapy (ART)	19 (15.1)	52 (17.4)	53 (18.5)	
	Living with HIV, not on ART	5 (3.9)	7 (2.3)	0 (0)	
Mother to child transmission of HIV, n (%)		1/24 (4.2)	3/59 (5.1)	1/53 (1.9)	0.666
Mode of delivery, n (%)	Caesarean section	78 (61.9)	154 (51.5)	118 (41.1)	< 0.001
	Normal vertex delivery	48 (38.1)	145 (48.5)	169 (58.9)	
Place of delivery, n (%)	Inborn	97 (77)	230 (76.9)	249 (86.8)	< 0.001
	Outborn	29 (23)	69 (23.1)	38 (13.2)	
Prior antibiotic therapy, n (%)		114 (90.5)	103 (34.4)	NA	< 0.001
Co-existing medical conditions, n (%)	Hyaline membrane disease	89 (70.6)	145 (48.5)	125 (43.6)	N/A
	Congenital pneumonia	4 (3.2)	24 (8.0)	13 (4.5)	
	Neonatal encephalopathy	7 (5.6)	25 (8.4)	18 (6.3)	
	Necrotizing enterocolitis	10 (7.9)	17 (5.7)	6 (2.1)	
	Other medical conditions*	90 (71.2)	237 (79.3)	205 (71.5)	
Co-existing surgical[#] conditions, n (%)		14 (11.1)	20 (6.7)	1 (0.3)	< 0.001

*Other medical conditions included: neonatal jaundice, glucose instability, meconium aspiration, congenital syphilis, congenital cardiac anomalies; [#]Surgical conditions included: 24 babies with gastrointestinal tract anomalies (necrotizing enterocolitis, gastroschisis, omphalocoele, spontaneous intestinal perforation, Hirschsprung's disease, trachea-oesophageal fistula, imperforate anus, duodenal atresia and malrotation); 8 babies with central nervous system malformations (myelomeningocoele, encephalocoele) and 3 babies with other conditions (choanal atresia, posterior urethral valves and septic arthritis). NA = not applicable.

Figure 1. Participant recruitment (1 February-31 October 2018).

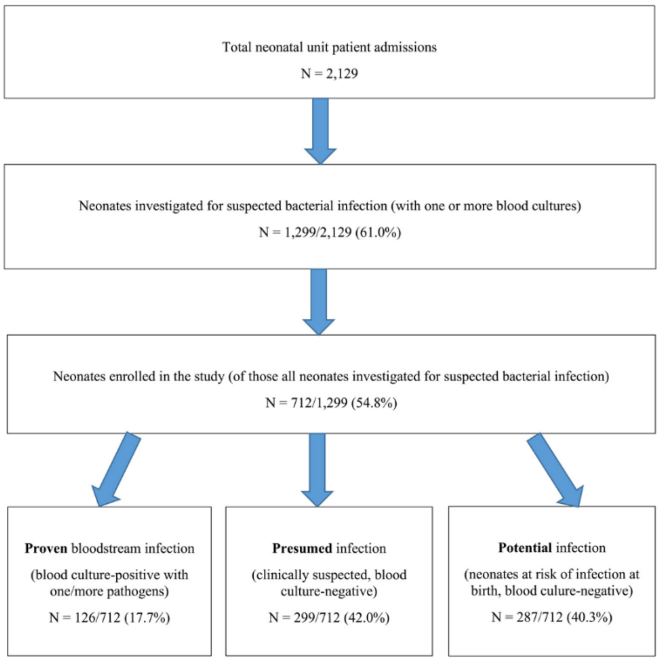
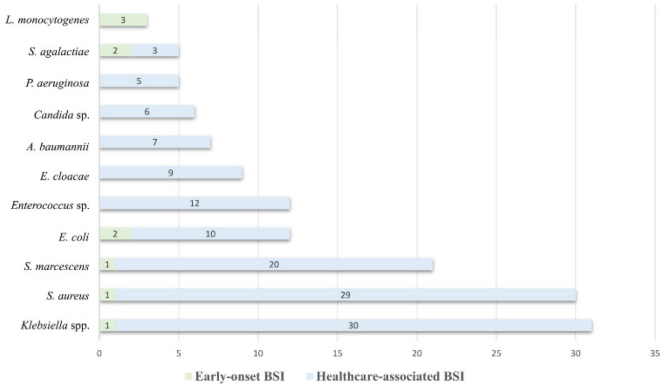


Figure 2. Timing of bloodstream infection episodes by pathogen type.



Of the 126 episodes of laboratory-confirmed BSI, blood cultures yielded 145 pathogens (111/126 [88%] from monomicrobial and 14/126 [12%] from polymicrobial BSI episodes); 11 blood cultures had 2 pathogens each and 4 had 3 pathogens each). Pathogen/s isolated for proven the BSI episodes included: *L. monocytogenes* (3); *S. agalactiae* (5); *Enterococcus* species (12); *S. aureus* (30); *Klebsiella* species (30); *S. marcescens* (21); *E. coli* (12); *E. cloacae* (9); *A. baumnannii* (7); *P. aeruginosa* (5); *C. freundii* (2); *S. paucimobilis* (1), *Pantoea* species (1); *C. perfringens* (1) and *Candida* species (4).

Table 2. Investigation, management and outcome of neonatal infection episodes (N = 712).

Infection episode variable		Proven bloodstream infection	Presumed infection	Potential infection	*p*-value
		blood culture-proven sepsis	clinical sepsis, blood culture-negative	asymptomatic neonate at risk of infection at birth	
		N = 126	N = 299	N = 287	
Day of life at blood culture collection, median (IQR)		8 (5-13)	1 (0-5)	0 (0-0)	< 0.001
Type of BSI episode, n (%)	Early-onset BSI	12 (9.5%)	-	-	-
	Hospital-acquired BSI	114 (90.5%)	-	-	
C-reactive protein^, median (IQR)		33 (3-74)	6.5 (1-21)	1 (1-2)	< 0.001
C-reactive protein^ ≥ 10 mg/dL, n (%)		80 (63.5%)	131 (43.8%)	15 (5.2%)	< 0.001
White cell count[&], median (IQR)		10.7 (5.6-15)	9.5 (6.9-14.6)	10.6 (8-15)	0.003
Platelet count, median (IQR)		191 (99-294)	227 (163-308)	245 (196-311)	0.146
Haemoglobin[&], median (IQR)		14.0 (12.0-16.5)	15.9 (14.1-17.9)	17.2 (15.5-19)	< 0.001
Duration of antibiotic therapy for the current infection episode, mean (SD)		6.9 (5.2)	5.2 (3.7)	3 (0.9)	< 0.001
Neonates requiring NICU admission, n (%)		55 (43.7)	71 (23.8)	20 (7)	< 0.001
Neonates requiring inotropic support, n (%)		20 (15.9)	13 (4.4)	3 (1.1)	< 0.001
Need for increased ventilatory support, n (%)	non-invasive ventilation	75 (59.5)	189 (63.2)	168 (58.5)	0.490
	invasive mechanical ventilation	36 (28.6)	59 (19.7)	19 (6.6)	< 0.001
	oscillatory ventilation	9 (7.1)	9 (3.0)	1 (0.4)	< 0.001
Neonates requiring a surgical procedure, n (%)		10 (7.9)	14 (4.7)	0 (0)	< 0.001
Empiric antimicrobial therapy of the current infection episode, n (%)	ampicillin + gentamicin	21 (16.6)	141 (47.1)	287 (100)	N/A
	piperacillin-tazobactam + amikacin	44 (34.9)	81 (27.1)	0 (0)	
	meropenem +- vancomycin	54 (42.9)	57 (19.1)	0 (0)	
	other regimen	7 (5.6)	20 (6.7)	0 (0)	
Outcome 30-days after blood culture collection, n (%)	alive at home	34 (27)	176 (58.9)	206 (71.8)	< 0.001
	still hospitalized	57 (45.2)	102 (34.1)	61 (21.3)	
	re-admitted#	1 (0.8)	6 (2)	5 (1.7)	
	lost to follow-up*	0 (0)	7 (2.3)	12 (4.2)	
	died	34 (27)	8 (2.7)	3 (1)	

^For babies at potential risk of infection at birth, CRP testing is performed at 24-48 hours of life, whereas babies with suspected infection at birth or during hospitalisation have CRP testing done at the same time as the blood culture. [&]The normal range for white blood cell count and haemoglobin values varies by gestational and postnatal age; clinicians applied the relevant cut-offs for the patient when interpreting these values. [#]Re-admitted = re-admitted to any hospital within 30-days of hospital discharge; *Lost to follow-up = unable to contact the participant's primary caregiver on day 30 post-enrolment for neonates that had been discharged/transferred. NA = not applicable.

Clinical and laboratory findings

Proven BSI episodes had onset at a median of 8 days (IQR = 5-13), whereas presumed infections occurred earlier (median 1, IQR = 0-5 days) ($p < 0.001$). Proven BSI episodes were associated with significantly higher CRP values than presumed or potential infections ($p < 0.001$) (Table 2). Among neonates at risk of potential infection, only 5.2% (15/287) had a CRP above the cut-off of 10mg/dL.

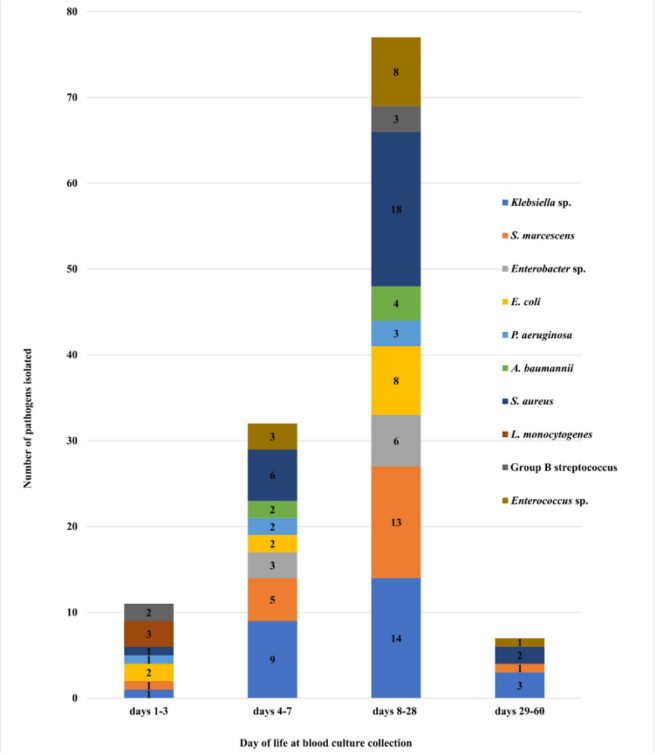
Among babies with early-onset BSI, 7/12 (58.3%) had maternal risk factors for infection (prolonged rupture of membranes, chorioamnionitis, and maternal urinary tract infection) and 5/12 (41.7%) had clinical signs/symptoms of infection (respiratory distress, glucose instability and thrombophlebitis). Early-onset BSI episodes (12/126; 9.5%) included typical maternally-derived pathogens such as Group B Streptococcus, *L. monocytogenes* and *E.coli*, as well as pathogens more commonly associated with HA-BSI (*K. pneumoniae, S. marcescens*, *S. aureus*).

Most proven BSI episodes were healthcare-associated (114/126; 90.5%), with a predominance of *Klebsiella pneumoniae* and *Staphylococcus aureus* (Figure 2). The spectrum of BSI pathogens fluctuated with post-natal age: *K. pneumoniae* occurred most frequently between days 3-10, and *S. aureus* and Candida species occurred most commonly after day 14 of life (Figure 3). Almost half of Gram-negative BSI pathogens were multi-drug resistant (39/80; 48.8%) including: *E. cloacae* [3/9; 33.3%]; *S. marcescens* [4/21; 19.0%]; *K. pneumoniae* [25/31; 80.6%], *E. coli* [1/12; 8.3%]; *A. baumannii* [6/7; 85.7%]. Two-thirds of *S. aureus* BSI were methicillin-resistant [20/30; 66.7%] and one-third of candidaemia episodes were fluconazole-resistant [2/6; 33.3%].

Antibiotic exposure and management

Prior courses of antibiotic therapy (unrelated to the current infection episode) were frequent in both the presumed infection (103/299; 34.4%) and the proven BSI groups (114/126; 90.5%). For empiric therapy of neonates in the potential infection group, the mean duration of ampicillin and gentamicin was 3.0 (SD = 0.9) days. Mean duration of antibiotic therapy was 5.2

Figure 3. Timing of bloodstream infection episodes for the top ten bloodstream infection pathogens.



For the top ten bloodstream infection pathogens, day of life at blood culture collection was categorised into four groups (day1-3; day 4-7; day 8-28 and day 29-60).

Table 3. Factors associated with mortality from laboratory-confirmed bloodstream infection.

Variable assessed	Univariate analysis *p*-value	Multivariate analysis *p*-value	Adjusted Odds ratio	95% CI
Factors associated with mortality				
Gender (male)	0.467	NS		
Length of stay prior to BSI onset (> 7 days)	0.253	NS		
Type of BSI episode (hospital-acquired)	0.602	NS		
Gestational age (< 32 weeks)	0.046	0.095	4.77	0.76-29.94
Birth weight (< 1,500g)	0.063	0.820	0.84	0.20-3.63
Type of BSI pathogen (Gram-negative)	0.023	0.023	3.23	1.17-8.92
Type of BSI (polymicrobial)	0.226	NS		
Antibiotic susceptibility profile (resistant)	0.720	NS		
Antibiotic therapy (discordant)	0.554	NS		

To determine factors associated with development of laboratory-confirmed BSI and mortality, intelligent multivariable logistic regression analysis was performed. All variables with $p < 0.1$ on univariate analysis were entered into the models. A p-value below 0.05 was considered statistically significant. NS= not significant.

(SD = 3.7) days for presumed infection and 6.9 (SD = 5.2) days for proven BSI, with a diverse range of empiric antibiotic regimens prescribed (Table 2). Of 12 neonates with early-onset, proven BSI, 5 (41.7%) required escalation of antibiotic therapy as the causative pathogen was not susceptible to empiric ampicillin plus gentamicin. Of the empiric antibiotic therapy prescribed for neonates with HA-BSI, 78/114 (68.4%) required no change to therapy, 21/114 (18.4%) had therapy de-escalated and 15/114 (13.2%) required escalation of therapy (e.g. addition of amphotericin B, colistin, linezolid, meropenem or vancomycin) following pathogen identification and susceptibility testing.

Clinical outcomes

The clinical impact of proven BSI was severe with substantially more neonates requiring admission to NICU, escalation of ventilatory and inotropic support, and surgical procedures, than neonates with presumed and potential infections (all $p < 0.001$) (Table 2). A total of 45/712 (6.3%) neonates died within 30 days of blood culture collection including 34/126 (27%) with proven BSI, 8/299 (2.7%) with presumed sepsis, and 3/287 (1%) with potential infection (Table 2). The relative risk of mortality at 30 days post-infection onset was significantly higher for neonates with proven BSI (3.99 [95%CI 3.31-4.81]) and presumed infection (1.76 [95% CI 1.38-2.25]) than for those with potential risk of infection (both $p < 0.001$).

All 4 neonates with early-onset BSI who demised (2 with Group B streptococcus, 2 with *L. monocytogenes*) were symptomatic at birth and died within 24 hours of blood culture submission despite NICU care. Most deaths among babies with HA-BSI were associated with Gram-negative pathogens (27/30; 90%) and were BSI-attributable (25/30; 83.3% occurring within 72 hours of blood culture collection). The median time from blood culture collection to death in babies with HA-BSI was 1 (IQR 0-2) day. BSI pathogen type (Gram-negative) was significantly associated with mortality (OR = 3.23; 95% CI = 1.17-8.92) on multivariable analysis (Table 3). However, there was no association between the appropriateness of therapy (concordance between the pathogen and the empiric antibiotic given) and outcome for Gram-negative HA-BSI. Case fatality rates (CFR) were highest for the following Gram-negative BSI pathogens (overall CFR 29/85 [34.1%]) : *P. aeruginosa* (4/5, 80%), *E. cloacae* (5/9, 55.6%), *S. marcescens* (9/21, 42.9%), *K. pneumoniae* (6/31, 19.4%), *A. baumannii* (2/7, 28.6%) and *E.coli* (3/12, 25%).

Discussion

In this study we prospectively characterised potential, presumed and proven neonatal BSI [5], documenting pathogen and antimicrobial susceptibility profile, infection impact and outcome. Neonates with proven BSI were significantly more likely to be preterm, of low birth weight and have co-existing surgical conditions. Although proven BSI episodes were less frequent than potential or presumed infections, they were more likely to be healthcare-associated, antimicrobial resistant and to lead to clinical deterioration and death. Gram-negative BSI was associated with a 3-fold increased risk of death, regardless of whether empiric antibiotic therapy was concordant or discordant.

Less than 20% of neonates evaluated for infection in this study had proven BSI, in keeping with blood culture yields reported from other Sub-Saharan Africa locations [25-28]. Proven BSI was however associated with significantly more abnormal laboratory markers of infection and a greater requirement for respiratory and inotropic support. Despite NICU admission and maximal organ support, neonates with proven BSI experienced high crude mortality rates (27%), comparable to that reported from other African country cohorts with similar proportions of preterm neonates [17,18,27,28]. Notably, most patients with proven BSI demised soon after blood culture collection (BSI-attributable mortality), highlighting the rapid clinical progression of bacteraemia, particularly in preterm neonates. Infection due to a Gram-negative pathogen was the single most important factor predicting neonatal mortality on multivariate analysis. Although *K. pneumoniae* was the leading Gram-negative pathogen, *S. marcescens* BSI had a higher CFR, in keeping with a report from a Johannesburg neonatal unit where the CFR for *S. marcescens* BSI was 55% [17].

Most BSI pathogens in this study exhibited substantial AMR, as described from other African neonatal units [12,15,17,18]. Efforts to ensure greater "bug-drug" concordance or appropriate initial antibiotic therapy of neonatal BSI are crucial [3,25], given increasing burden of Gram-negative and AMR pathogens in early-onset BSI and HA-BSI in African neonatal units. In this cohort, > 40% of early-onset infections required a change to empiric therapy to provide appropriate coverage for the invasive pathogen, whereas < 15% of HA-BSI episodes had discordant empiric antibiotic therapy. This finding underscores the need to regularly review local BSI pathogen and AMR trends to inform empiric antibiotic recommedations.

For African neonatal units that lack microbiology services, analysis of pooled regional neonatal BSI data may facilitate developing data-driven empiric antibiotic recommendations.

Some risk factors for developing neonatal BSI are difficult to modify. Our study highlighted the role of prematurity, underlying surgical conditions and prolonged hospitalisation as risk factors for neonatal infection. Notably, in this cohort > 90% of all BSI episodes were healthcare-associated and one-third of neonates remained hospitalised at the 30-day study outcome assessment. To modulate infection risk in hospitalised neonates, novel interventions that interrupt the acquisition and invasion of pathogenic flora may be required to prevent BSI including interventions that reduce disruption of skin and gut barriers and delay colonisation by Gram-negative pathogens. African neonatal units should focus on implementing effective infection surveillance, infection prevention and antibiotic stewardship to reduce infection burden and preventable neonatal deaths [6].

More than 80% of neonates evaluated for possible bacterial infection in this cohort had a negative blood culture despite having maternal risk factors for infection at birth and/or signs and symptoms suggestive of infection at birth or during hospitalisation. Empiric treatment of presumed and potential infections contributes substantially to the high rates of antibiotic prescribing in virtually all neonatal units. More sensitive point-of-care tests to rule-in/rule-out bacterial infection and rapidly identify neonatal pathogens are needed. The routine use of CRP in our neonatal unit facilitates antibiotic stewardship by allowing for prompt antibiotic discontinuation in most neonates with risk factors for potential infection. In this cohort, neonates with presumed infection appropriately received longer duration of antibiotic therapy than those with potential infection (most were clinically unwell, had raised CRPs and required new or increased respiratory support). The outcome of neonates with potential infection risk and presumed infections in this study was generally favourable.

Limitations of this study include the single site, inclusion of only half of the potentially eligible neonates owing to convenience sampling with non-availability of parents to grant informed consent, and the lack of long-term neurodevelopmental follow-up of BSI survivors. Strengths of the study include the inclusion of all infection categories (proven, presumed and potential) and the close clinical follow-up with 30-day post-infection outcome data.

Future studies in African neonatal units should report both laboratory-confirmed and clinically suspected, culture-negative infection episodes, as these are major drivers of antibiotic use. Regional or country-level data on the pathogen and AMR profile of early-onset and HA-BSI in hospitalised neonates is critically-needed in Africa. National ministries of health should use available neonatal BSI data to identify and target regions with high infection burden for quality improvement interventions, and for development of data-driven empiric antibiotic recommendations. A renewed focus on neonatal unit infection surveillance, infection prevention and antibiotic stewardship, will contribute to improved outcomes for small and sick newborns in Africa.

Conclusions

Most neonates evaluated for suspected infection had potential and presumed (culture-negative) infection, with favourable 30-day outcomes. Proven BSI episodes were predominantly healthcare-associated and antimicrobial resistant infections. Neonates with proven BSI had high case fatality rates; odds of mortality increased 3-fold for neonates with BSI episodes caused by Gram-negative pathogens.

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Authors' contributions

AD, AB, MFC, ACW and SC conceptualised the study. AD collected and analysed the data and prepared the first draft. All authors read, edited and approved the final manuscript.

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