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Original article

Maternal colonization and early-onset neonatal bacterial sepsis in the Gambia, West Africa: a genomic analysis of vertical transmission

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ABSTRACT

Objectives: To define bacterial aetiology of neonatal sepsis and estimate the prevalence of neonatal infection from maternal genital tract bacterial carriage among mother-newborn pairs.
Methods: We carried out a cross-sectional study of newborns with clinical sepsis admitted to three hospitals in the Gambia neonatal wards. Neonatal blood cultures and maternal genital swabs were obtained at recruitment. We used whole-genome sequencing to explore vertical transmission for neonates with microbiologically confirmed bloodstream infection by comparing phenotypically-matched paired neonatal blood cultures and maternal genital tract bacterial isolates.
Results: We enrolled 203 maternal-newborn pairs. Two-thirds (67%; 137/203) of neonates presented with early-onset sepsis (days 0–6 after birth) of which 26% (36/137) were because of a clinically-significant bacterial pathogen. Blood culture isolates from newborns with early-onset sepsis because of *Staphylococcus aureus* ($n = 5$), *Klebsiella pneumonia* ($n = 2$), and *Enterococcus faecalis* ($n = 1$), phenotypically matched their maternal genital tract isolates. Pairwise single-nucleotide variants comparisons showed differences of 12 to 52 single-nucleotide variants only between maternal and newborn *S. aureus* isolates, presumably representing vertical transmission with a transmission rate of 14% (5/36).
Conclusions: We found a low prevalence of vertical transmission of maternal genital tract colonization in maternal-newborn pairs for early-onset neonatal sepsis in the West African context. Identifying infection acquisition pathways among newborns is essential to prioritize preventive interventions, which could be targeted at the mother or infection control in the hospital environment, depending on the major pathways of transmission. **Uduak A. Okomo, Clin Microbiol Infect 2023;29:386.e1–386.e9**

Introduction

Infections are among the leading causes of newborn deaths globally and are more prevalent in resource-limited settings [1]. In sub-Saharan Africa, infections account for nearly one-quarter of neonatal deaths [2]. Early-onset neonatal bacterial sepsis occurring

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day 0 to 6 after birth is often associated with vertical transmission of infection, occurring shortly before or during labour. In resource-limited settings, early-onset infections may include infections acquired horizontally from environmental (home or hospital) sources at birth, with lower hygiene measures during delivery and initial care of the baby [3]. Late-onset (7–27 days after birth) neonatal sepsis is mostly horizontally acquired.

Bacterial flora diversity of the female lower genital tract can change in response to endogenous and exogenous influences, including age and pregnancy and is best characterized using culture-independent molecular approaches, including high-throughput sequencing and metagenomics [4,5]. Vertical transmission of bacterial pathogens from the maternal lower genital tract has traditionally been studied using conventional culture-dependent techniques, such as serotyping, and antimicrobial susceptibility, to compare bacterial isolates from newborn surface contamination and invasive disease, with paired maternal rectovaginal isolates [6,7]. Microbiologic techniques, however, lack sufficient discriminatory power to adequately delineate vertical from horizontal routes of bacterial transmission to the newborn. Molecular and genomic typing of bacterial pathogens complements culture-based techniques by providing appropriate discriminatory analyses to detect transmission events and the relatedness of strains. Whole-genome sequencing (WGS) has been used to demonstrate the vertical transmission of maternal group B Streptococcal (GBS) infection in mother-newborn pairs for both surface contamination and perinatal disease [8]. WGS has also been used to identify and define transmission pathways of GBS [9] and *Staphylococcus aureus* outbreaks in neonatal units [10,11]. In this study, we combined traditional bacteriologic culture with WGS to assess vertical transmission of maternal colonization in maternal-newborn dyads with neonatal sepsis in a West African resource-limited setting.

Methods

Ethics approval

This study was approved by The Gambian government/MRC Gambia Joint Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee. Mothers/caregivers of all newborn participants gave written informed consent. We followed STROBE-NI recommendations for reporting observational studies on neonatal infections [12].

Study design and setting

This was a secondary analysis of prospectively collected and archived bacterial isolates from mother-newborn pairs that were part of a hospital-based case-control study of invasive neonatal infections. The study was carried out at the Edward Francis Small Teaching Hospital, Banjul and Kanifing General Hospital, and the postnatal ward of Brikama District Hospital over a period of 17 months (April–August 2015 [Edward Francis Small Teaching Hospital only] and February 2016–January 2017 [all three facilities]). These main public hospitals serve a total population of 1.1 million people (59% of the national population) [13] Over three-quarters of women in the region delivered in a health facility, with the largest number occurring at Edward Francis Small Teaching Hospital (~6000 deliveries per year), followed by Brikama District Hospital (~5000 deliveries per year), and Kanifing General Hospital (~3000 deliveries per year). National neonatal mortality, stillbirth, and preterm birth rates are 26 per 1000 live births, 22 per 1000 births, and 12 per 100 live births, respectively [14].

Participants and procedures

Inclusion criteria for neonates were postnatal age 0 to 27 days (day 0 being the day of birth), presentation with one or more clinical signs of possible serious bacterial infection (Table S1), and admission weight of 1000 g or more. Mothers of eligible neonates were residents in the study area and presented with documented evidence of having attended at least one antenatal care visit. For all eligible neonatal admissions, peripheral blood was sampled (1.0–1.5 mL) drawn following a strict aseptic technique [15], before neonatal antibiotic treatment (or within 12 hours for overnight admissions). Supportive care and antibiotic treatment were provided as per the national protocol (Table S2). We took rectovaginal swabs from all consenting mothers at the time of neonatal recruitment. A small flocked swab (Copan Diagnostics, Brescia, Italy) was used to wipe the lower third of the vaginal and anal surface mucosa according to standard procedures [16]. Mothers could withhold consent for having their samples collected to permit their infants to be studied. All samples were processed at the MRC Gambia at London School of Hygiene and Tropical Medicine ISO 15189 accredited Clinical Microbiology laboratory and Genomics Core Facility.

Laboratory methods

Bacterial culture

We used automated blood culture (BACTEC 9050) to isolate pathogens. Bacterial strains from cultures were identified with conventional biochemical tests and the API 20E strip test (bio-Merieux-Vitek, Hazelwood, MO, USA). We classified blood culture isolates as clinically significant or clinically non-significant (Table S3) [17,18]. Rectovaginal swabs were placed into skim-milk tryptone-glucose-glycerol transport medium, refrigerated, transported in cool containers to the MRC Gambia at London School of Hygiene and Tropical Medicine Clinical Microbiology laboratory and processed by standard methods including subculture on solid media as follows: positive blood cultures (blood, chocolate, and MacConkey agar); rectovaginal swabs (blood agar and MacConkey agar). Priority was given to the identification of known bacterial pathogens associated with neonatal sepsis and meningitis. For each sample up to three morphological similar isolates were sub-cultured for susceptibility testing and further storage; we did not consider the presence of multiple species and strains. Phenotypic antimicrobial sensitivity testing was carried out using the Kirby-Bauer disc diffusion method with Oxoid antimicrobial susceptibility discs (Thermo Scientific, Waltham, MA) for a panel of antibiotics that are used locally by 2016 Clinical and Laboratory Standards Institute guidelines (Table S4).

Bacterial WGS

Bacterial isolates were frozen in 1 ml vials and stored at −80 °C before subculture onto a blood agar plate for 24–48 h, followed by DNA extraction from a single pure colony using a commercial kit. Sequencing was performed on Illumina MiSeq (Illumina, San Diego, California, USA) using the NEBNext® Ultra™ libraries and protocols (New England Biolabs, UK). Sequence reads were trimmed, and assemblies generated using SPAdes (version 3.13.0), with kmer sizes 21, 33, 55, and 77, and annotated using Prokka v1.13 [19,20]. Single-nucleotide variants (SNVs) were determined using Snippy v4.3.6 with the following references: *Escherichia coli* str. K-12 MG1655, *Staphylococcus aureus* str. NTC 8325, *Klebsiella pneumoniae* str. HS11286 and *Enterococcus faecalis* str. V583. Core genome analyses were done using Roary v3.12.0 [21]. Sequence data were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject PRJNA723854 (for isolate accessions see Table S5).

Outcomes

We defined three categories of neonatal sepsis: (a) blood culture-proven bacterial sepsis with a clinically-significant pathogen; (b) blood culture-proven bacterial sepsis with a clinically non-significant pathogen; and (iii) clinical (culture-negative) sepsis. We defined early-onset sepsis as occurring on days 0 to 6 after birth (day 0 as day of birth) and late-onset sepsis as that on days 7 to 27 after birth.

We defined maternal colonization as a positive rectovaginal bacterial culture (*S. aureus*, *K. pneumoniae*, *E. coli*, Group B *Streptococcus*, Group A *Streptococcus*, *Pseudomonas* spp., Citrobacter spp., Proteus spp., and *Acinetobacter* spp.) without signs or symptoms of infection, as these bacteria are known to cause infections in neonates. Neonatal blood culture and maternal rectovaginal isolates were considered phenotypically-matched if both were identical species and antibiogram.

Statistical analysis

Within the study sample of sick neonates, we compared descriptive data between groups based on blood culture results. Categorical and continuous descriptive variables were compared by χ^2 and Mann–Whitney *U* tests respectively. Analyses were performed using STATA v16 (StataCorp, College Station, TX, USA).

Results

We enrolled 203 mother-newborn pairs including 202 newborns with blood culture (Fig. 1). Bacteria were isolated from the blood of 45% (91/203) of neonates; 25% (51/203) of all cultures grew a clinically-significant bacterial pathogen and 20% (40/203) grew a clinically non-significant pathogen. Two-thirds (67%; 137/203) of all cases presented as early-onset sepsis (days 0–6 after birth) of

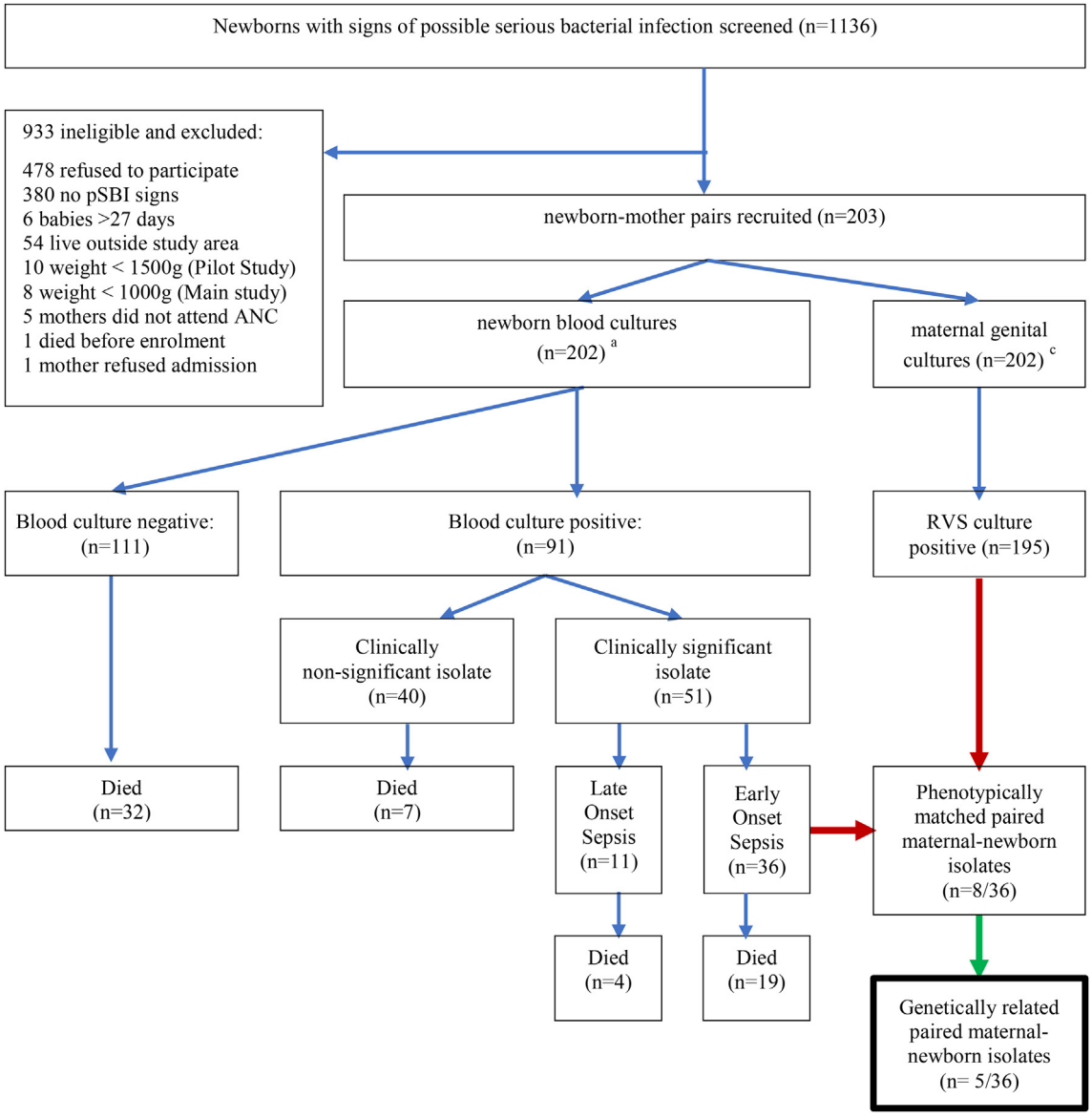

Fig. 1. Flow chart of participant recruitment.

which one quarter (26%; 36/137) were due to a clinically-significant bacterial pathogen (Table 1). Sixty-two (30%) neonates died; 56 neonates died on or before 27 days postnatal age (three of which died at home after discharge from the hospital) and six died after 27 days postnatal age. Overall, neonates with blood cultures positive for a clinically-significant pathogen had a higher case fatality compared to those with negative cultures and those with cultures positive for a clinically non-significant pathogen (45% [23/51] *vs* 29% [32/111] *vs* 18% [7/40]; $P = 0.02$).

S. aureus was the predominant species isolated, accounting for 6% (8/137) of cases of early-onset neonatal sepsis and 8% (5/66) of late-onset sepsis cases (Table 2). One infant had a polymicrobial culture with *E. coli* and *Enterobacter* spp. Fig. 2 shows the distribution of clinically-significant pathogens in the first week (days

Table 1
Characteristics of neonates and mothers stratified by neonatal blood culture result[a]

Characteristics	All newborns with suspected bacterial sepsis ($N = 203$)	Culture-confirmed bacterial sepsis with a clinically-significant pathogen ($N = 51$)[b]	Culture-confirmed bacterial sepsis with a clinically non-significant pathogen (N = 40)[b]	Clinical sepsis ($N = 111$)[b]	p[e]
Neonatal					
Sex					
Male	123 (61)	33 (65)	24 (60)	65 (59)	0.76
Female	80 (39)	18 (35)	16 (40)	46 (41)	
Median postnatal age in d (IQR)	3 (1–9)	2 (1–8)	2 (1–7)	3 (1–10)	
0–6 d	137 (67)	36 (71)	29 (73)	72 (65)	0.60
7–27 d	66 (33)	15 (29)	11 (27)	39 (35)	
Median gestational age in weeks (IQR)	38 (36–39)	38 (36–40)	38 (36–39)	38 (36–39)	
Preterm (<37 wks)	50 (26)	12/47 (26)	10/39 (26)	28/106 (26)	0.10
Full term (≥37 wks)	143 (74)	37/47 (74)	29/39 (74)	78/106 (74)	
Median birthweight in kg (IQR)	3000 (2600–3400)	3000 (2500–3400)	3000 (2800–3200)	3000 (2600–3400)	
Low birth weight <2500 g	33 (18)	7/48 (15)	3/38 (8)	23/101 (23)	0.10
Normal birth weight ≥2500 g	155 (82)	41/48 (85)	35/38 (92)	78/101 (77)	
Birth location					
Health facility	184 (91)	48 (94)	37 (93%)	99 (89%)	0.64
Home/CBC	19 (9)	3 (6)	3 (7)	12 (11)	
Mode of delivery					
Vaginal delivery[c]	178 (88)	47 (92)	38 (95)	92 (83)	0.10
Caesarean section	25 (12)	4 (8)	2 (5)	19 (17)	
Resuscitation at delivery	58 (32)	16 (32)	14 (40)	28 (29)	
Pre-recruitment antibiotic exposure	34 (17)	10 (20)	8 (20)	16 (14)	0.60
Median length of admission, days (IQR)	6 (3–9)	6 (2–9)	6.5 (4–9.5)	6 (3–9)	0.54
Clinical signs of pSBI					
Fast breathing (%)	103 (51)	34 (67)	22 (55)	46 (41)	0.01-
Severe chest indrawing (%)	42 (21)	14 (27)	6 (15)	21 (19)	0,30
Feeding problems (%)	79 (39)	19 (37)	17 (43)	43 (39)	0.20-
Fever (%)	106 (52)	22 (43)	17 (43)	66 (59)	0.06
Hypothermia (%)	30 (15)	9 (18)	5 (13)	16 (14)	0.78
Lethargy or unconsciousness (%)	30 (15)	16 (31)	6 (15)	14 (13)	0.01
Reported or observed convulsions (%)	52 (26)	14 (27)	14 (35)	24 (22)	0.24
Outcome					
Died by d 27 of life[d]	56 (30)	21 (41)	6 (15)	29 (26)	0.03
Died overall	62 (31)	23 (45)	7 (18)	32 (29)	0.02
Maternal					
Median age at delivery, yrs (IQR)	27 (22–32)	27 (24–32)	27 (23–32)	27 (22–32)	0.65
Education					
Some education (ever attended school)	145 (71)	37 (73)	30 (75)	77 (69)	0.78
No education	58 (29)	14 (27)	10 (25)	34 (31)	
Parity					
Multiparous	141 (69)	39 (76)	26 (65%)	75 (68%)	0.04
Primiparous	62 (31%)	12 (24)	14 (35)	36 (32)	
Fever before or during labour (by recall)	52 (26)	16 (31)	11 (28)	25 (23)	0.47
Intrapartum antibiotic exposure (by recall)	4 (2)	1 (2)	1 (3)	2 (2)	0.90
Genital tract bacterial carriage	195 (97)	51 (100)	35 (88)	108/110 (98)	0.01

IQR, interquartile range; CBC, Community Birth Companion.
[a] Excluding one infant who did not have a blood culture.
[b] Denominators (X/Y) are presented for variables with missing data or otherwise indicated.
[c] Included both unassisted and assisted (forceps, vacuum extraction) vaginal deliveries.
[d] Fifty six neonates died on or before 27 days postnatal age, three of which died at home after discharge from hospital, whereas six died after 27 days postnatal age.
[e] p values are χ^2 or Fisher exact, or Kruskal-Wallis (for medians) where appropriate, excluding missing values.

Table 2
Distribution of isolated bacterial pathogens stratified by neonatal characteristics

Blood culture result	Overall prevalence (n = 203)	Prevalence within different age groups (%)		Prevalence by birth location (%)		Gestational age <37 wks (%) ($n = 50$)[a]	Prevalence by mode of delivery (%)		Died (%) ($n = 62$)[c]
		Aged 0–6 d ($n = 137$)	Aged 7–27 d ($n = 66$)	Hospital ($n = 184$)	Home ($n = 18$)		Vaginal ($n = 177$)[b]	Caesarean ($n = 25$)	
Any positive culture	91 (45)	65 (47)	26 (39)	85 (46)	6 (33)	22 (44)	85 (48)	6 (24)	30 (48)
Clinically-significant pathogen									
Staphylococcus aureus	13 (6)	8 (6)	5 (8)	12 (7)	1 (5)	2 (4)	13 (7)	0	3 (5)
Burkholderia cepacia	9 (4)	8 (6)	1 (2)	9 (5)	0	0	9 (5)	0	5 (8)
Klebsiella pneumoniae	8 (4)	7 (5)	1 (2)	8 (4)	0	7 (14)	6 (3)	2 (8)	4 (6)
Klebsiella oxytoca	1 (<1)	1 (1)	0	1 (<1)	0	0	0	1 (4)	0
Pseudomonas luteola	6 (3)	4 (3)	2 (3)	6 (3)	0	0	6 (3)	0	4 (6)
Pseudomonas species	1 (<1)	0	1 (2)	1 (<1)	0	0	1 (<1)	0	0
Enterococcus faecalis	3 (1)	3 (2)	0	3 (2)	0	1 (2)	3 (2)	0	2 (3)
Acinetobacter baumanii	2 (1)	2 (1)	0	2 (1)	0	1 (2)	2 (1)	0	1 (2)
Escherichia coli[d]	2 (1)	0	2 (3)	2 (1)	0	1 (2)	2 (1)	0	2 (3)
Streptococcus species	2 (1)	2 (1)	0	2 (1)	0	0	2 (1)	0	1 (2)
Achromobacter xylosoxidans	1 (<1)	0	1 (2)	1 (<1)	0	0	1 (<1)	0	0
Citrobacter species	1 (<1)	0	1 (2)	0	1 (5)	0	1 (<1)	0	1 (2)
Salmonella species	1 (<1)	0	1 (2)	0	1 (5)	0	1 (<1)	0	0
Pantoea species	1 (<1)	1 (1)	0	1 (<1)	0	0	0	1 (4)	0
Clinically non-significant pathogen									
Coagulase-negative staphylococci	32 (16)	22 (16)	10 (15)	30 (16)	2 (11)	9 (18)	31 (17)	1 (4)	4 (6)
Viridans streptococci	2 (1)	2 (1)	0	2 (1)	0	0	2 (1)	0	1 (2)
Micrococcus species	2 (1)	2 (1)	0	2 (1)	0	0	2 (1)	0	2 (3)
Bacillus species	4 (2)	3 (2)	1 (2)	3 (2)	1 (5)	1 (2)	3 (2)	1 (4)	0

[a] Ten babies had missing data for gestational age.
[b] Includes spontaneous (172) and assisted (vacuum [3] and breech [2]) vaginal deliveries.
[c] Fifty nine neonates died on admission, 3 died at home after discharge.
[d] One baby had polymicrobial culture with Enterobacter cloacae.

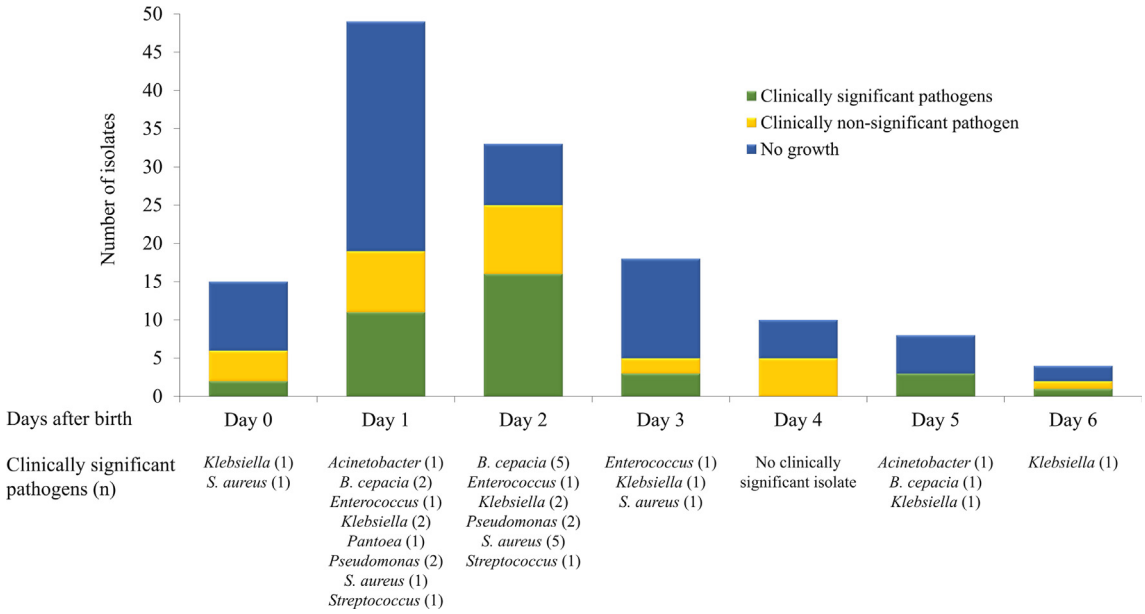

Fig. 2. Distribution of blood culture results and clinically-significant bacterial isolates by postnatal age at diagnosis.

0–6). Among *Klebsiella* isolates, non-susceptibility to World Health Organization-recommended first-line gentamicin was 89% (8/9) with non-susceptibility to World Health Organization-recommended second-line therapy (third-generation cephalosporins) ranging from 67% to 100% (Table S6).

We obtained genital tract cultures from all but one of the infant mothers enrolled in the study. Most (97%) mothers were colonized with at least one potentially pathogenic organism (Table S7). Eight (22%) of 36 neonates with Early-onset neontal sepsis (EONS) due to a clinically-significant bacterial pathogen were born to mothers colonized with a phenotypically matched isolated: five with *S. aureus* sepsis; two with *K. pneumoniae* sepsis and one with sepsis due to *E. faecalis* (Table 3). Both maternal-newborn Klebsiella pairs were emergency caesarean deliveries and were highly divergent [>15 000 SNVs with different STs and species). Genomic analysis revealed both neonatal isolates (ST1535) to be *Klebsiella quasipneumoniae subspecies similipneumoniae*, which along with isolates of another six cases of *K. pneumoniae* (ST 39) sepsis reported here, were identical to *Klebsiella* ST 1535 and ST 39 isolates from a previously reported outbreak of multidrug-resistant ESBL–producing *Klebsiella* sepsis in one of the neonatal wards [22]. These cases were subsequently excluded from further analyses. All *S. aureus* isolates were methicillin sensitive. Pairwise SNV comparisons between maternal and newborn isolates showed differences of 12 to 52 SNVs, presumably representing vertical transmission with a transmission rate of 14% (5/36). Two of the maternal-newborn *S. aureus* pairs were the same ST (ST 627); however, neonatal isolates from each pair differed by 82 SNVs, possibly reflecting two unrelated occurrences of ST 627 in their mothers given the lack of epidemiologic links in time and place (delivered at different health care facilities and admitted 21 days apart). Paired maternal and newborn *E. faecalis* isolates were the same ST but differed by 108 SNVs.

Discussion

Neonatal infections remain an important challenge for child survival and health worldwide. Our understanding of the routes of transmission remains incomplete; yet is critical to developing research priorities and appropriate strategies for prevention. Here, for the first time from a West African setting, we present a comparative genomic analysis of paired maternal and neonatal isolates to evaluate mother-to-newborn transmission events among neonates with culture-confirmed early-onset bacterial sepsis. We found a lower prevalence of vertical transmission of maternal bacterial genital tract colonization for early-onset neonatal sepsis in only 14% (5/36) of neonates, with no genetically near-identical pairs (0 SNVs).

A systematic review of vertical transmission of early-onset neonatal infection showed that only 1.1% (95% CI 0.2–2.0) of newborns of colonized mothers not exposed to intrapartum antibiotics developed laboratory-confirmed bacterial infection [23]. Most studies included in the review focused on maternal GBS colonization and were from high-income countries. In a more recent GBS-specific review, the risk of early-onset GBS disease was 1.1% (95% CI 0.6%–1.5%) for newborns born to women colonized with GBS in pregnancy in settings without a policy for providing intrapartum antibiotic prophylaxis for positive GBS screening. Stratified by region, the risk was reported to be lower in Africa (0.7%; 95% CI 0.3%–1.1%) than in Europe and America (1.34%; 95% CI 0.7–2.0) [24] Although invasive bacterial disease risk in neonates of colonized mothers may be low, in the neonates that do develop early-onset sepsis, the organism may have been part of the maternal vaginal flora. However, a previous study in Uganda found no concordance between organisms recovered from newborn blood and maternal vaginal cultures in the mother-newborn pairs [6]. These data were generated through conventional culture methods rather than genomic approaches used in our study and differed regarding the site of maternal swab collection (high or low vaginal swab, rectum, or perianal region), the timing of swab collection (during pregnancy), and laboratory methods. Our finding of few confirmed instances of vertical transmission of early-onset neonatal sepsis among colonized mothers might contribute to evidence on why intrapartum antibiotic prophylaxis and other strategies such as chlorhexidine intravaginal and neonatal wipes have not been highly effective for preventing neonatal sepsis in sub- Saharan Africa [25].

In concordance with previous data from West Africa, *S. aureus* was the predominant cause of early-onset neonatal sepsis in our

Table 3
Genomic relatedness of phenotypically-matched paired maternal rectovaginal and neonatal blood culture isolates

Pair	Strain	Organism	Age at culture	GA (wks)	Birth weight (g)	Place of birth	Mode of delivery	Maternal intrapartum antibiotic exposure	Neonatal outcome	MLST	Pairwise SNP distance	Comments
Early-onset sepsis												
EOS-Pair 1	Maternal	*S. aureus*	28 yrs			Brikama district Hospital	SVD	No		ST 627	12	
	Neonatal	*S. aureus*	2 d	38	2800				Alive	ST 627		
EOS-Pair 2	Maternal	*S. aureus*	26 yrs			SOS Clinic (NGO facility)	SVD	No		ST 627	14	
	Neonatal	*S. aureus*	1 d	40	3300				Alive	ST 627		
EOS-Pair 3	Maternal	*S. aureus*	26 yrs			Banjulinding Health Centre	SVD	No		Novel	30	
	Neonatal	*S. aureus*	0 d	38	2400				Died	Novel		
EOS-Pair 4	Maternal	*S. aureus*	26 yrs			Bakau Health Centre	SVD	No		ST 15	52	
	Neonatal	*S. aureus*	2 d	42	3800				Died	ST 15		
EOS-Pair 5	Maternal	*S. aureus*	23 yrs			Banjulinding Health Centre	SVD	No		ST 6	41	
	Neonatal	*S. aureus*	2 d	40	2790				Alive	ST 6		
EOS-Pair 6	Maternal	*K. pneumoniae*	36 yrs			Mbowen Clinic (Private facility)	Emergency C/S	No		ST 15	15 159	Newborn isolates genetically identical to nosocomial outbreak strains
	Neonatal	*K. pneumoniae*	2 d	34	2500				Alive	ST 1535		
EOS-Pair 7	Maternal	*K. pneumoniae*	24 yrs			Mbowen Clinic (Private facility)	Emergency C/S	No		Unknown	42 896	Newborn isolates genetically identical to nosocomial outbreak strains
	Neonatal	*K. pneumoniae*	2 d	34	2500				Died	ST 1535		
EOS-Pair 8	Maternal	*E. faecalis*	23 yrs			Brikama district Hospital	SVD	No		646	108	
	Neonatal	*E. faecalis*	3 d	37	2500				Alive	646		
Late-onset sepsis												
LOS-Pair 1	Maternal	*S. aureus*	28 yrs			Home	SVD	No		ST 1	45 168	
	Neonatal	*S. aureus*	21 d	40	4500				Alive	ST 152		
LOS-Pair 2	Maternal	*E. coli*	17 yrs			Brikama district Hospital	SVD	No		ST 10	5801	
	Neonatal	*E. coli*	26 d	36	2600				Died	ST 10		
LOS-Pair 3	Maternal	*E. coli*	25 yrs			Pirang Health Centre	SVD	No		ST 1193	6044	
	Neonatal	*E. coli*	8 d	39	2400				Died	Unknown		

C/S, caesarean section; EOS, early-onset sepsis; GA, gestational age at birth; LOS, late-onset sepsis; NA, not applicable; SVD, spontaneous vaginal delivery.

setting [26], and the only organism demonstrated to be vertically transmitted. This is in sharp contrast to high-income country settings where perinatal vertical transmission of *S. aureus* is reportedly rare, with it rather being a leading cause of outbreaks and health care-associated infections in neonatal intensive care units [27]. In these settings, decolonization of colonized neonates and health care workers has been recommended to prevent transmission and infections because of methicillin-resistant *S. aureus* [28]. The adoption and success of decolonization in resource-limited settings are precluded by limited laboratory capacity for culture-based detection; the short interval between colonization to infection and high recolonization rates [27]. Outbreaks in hospitalized African neonates are predominantly because of Gram-negative bacteria [29].

Despite our genomic analyses, we found that most newborns with culture-positive early-onset sepsis in our cohort did not have a maternal linkage. This might be related to the fact that we only picked and sequenced single colonies, precluding the ability to account for within-host diversity and multi-strain colonization. It is possible that some mothers would have been colonized with multiple strains of the pathogenic bacteria, and in some cases by chance, may not have picked the colony for the strain that was passed to the baby; therefore, the absence of culture-positive transmission is not evidence for the absence of transmission. Because this study was designed to focus on vertical transmission, we were unable to explore sources elsewhere in the hospital environment, particularly the labour ward. Failures in the aseptic technique can lead to neonatal infections, including early onset [3].

Our study has strengths and limitations. Strengths include the epidemiologic design with mother-baby pairs, consistent case definitions and rigorous genomic methods. Our neonatal sepsis cohort was not population-based and not representative of all neonatal sepsis cases at participating facilities. Notably, very low birthweight babies were excluded because of challenges in obtaining sufficient samples, yet they are the most vulnerable. Even though a quarter of neonatal blood cultures were positive for a clinically-significant pathogen, our genomic data is limited to a small number of cases. Our definition of genetic relatedness was based on an arbitrary SVP cut-off as there is little agreement in the literature. However, the presence of a few (tens of) SNVs indicates isolates are closely related and increases the likelihood of arising from the same source, whereas the presence of many (hundreds or more) SNVs indicates that isolates are distantly related, implying differing reservoir populations [30]. This depends critically on the pathogen as well as the context—outbreak or nonoutbreak settings. Data on comparative genomic analyses of bacterial isolates from the maternal carriage and neonatal disease are available for GBS [8]. Evidence is however lacking for pathogen-specific genetic relatedness cut-offs for transmission in similar nonoutbreak settings. A recent study [31] attempted to define a genetic relatedness SNP cut-off between any two methicillin-resistant *S. aureus* lineages during an outbreak and proposed a conservative cut-off of 25 whole-genome SNPs or 15 core genome SNPs, above which 95% of recent methicillin-resistant *S. aureus* transmission events can be ruled out within a period of 6 months. A major limitation of SNP cut-offs is that they cannot be used to identify sources and recipients of transmission (directionality) or to establish the probability of transmission. Application of this threshold to our data would have further reduced the prevalence of likely vertical transmission.

In conclusion, neonatal infections remain an enormous issue in the Gambia, and we demonstrated a low prevalence of vertical transmission of maternal colonization for early-onset neonatal sepsis. Further context-specific research is required to better direct interventions aimed at reducing the burden of infection-specific neonatal mortality in sub-Saharan Africa, and importantly, hospital-acquired infections in newborn care units. One such approach is the use of next-generation sequencing technologies and metagenomic approaches to improve the characterization of maternal genital tract colonization, as well as complement surveillance of environmental contamination in hospital facilities.

Author contributions

U.O., J.E.L., and B.K. conceptualised the study. U.O. wrote the first draft of the manuscript and prepared all figures and tables. S.D., S.J., and N.K. carried out pathogen isolation and antimicrobial sensitivity testing with guidance and support by K.L.D. A.G., T.D., and M.G.J. provided patient care. A.A. and S.J. carried out DNA extraction and sequencing of isolates with technical and administrative support by A.K.S. S.Y.B. and performed the bioinformatic analysis. K.E.H. guided the interpretation of phenotypic and genomic analysis Statistical analysis was done by U.O., with advice from J.E.L. and B.K. and K.L.D. All authors provided input to the overall direction and content of the paper, and have seen and approved the final version.

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Transparency Declaration

No author declared a conflict of interest about the submitted work. UO reports grants from the MRC UK Research & Innovation (UKRI), United Kingdom, Wellcome, United Kingdom and The Thrasher Research Fund, United States outside the submitted work. KH reports numerous research grants outside the submitted work. BK reports grants from UKRI, Wellcome and National Institue of Health and Care Research (NIHR), United Kingdom for a variety of projects relating to vaccines and maternal and newborn health. The other authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.10.012.

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