

Causes of stillbirths among women from South Africa: a prospective, observational study

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Summary

Background About 2·6 million third-trimester stillbirths occur annually worldwide, mostly in low-income and middle-income countries, where the causes of these deaths are rarely investigated.

Methods We did a prospective, hospital-based, observational study in Soweto, South Africa, to investigate the causes of stillbirths in fetuses of at least 22 weeks' gestational age or with a birthweight of at least 500 g. Maternal clinical information was abstracted from medical records. Investigations included placental macroscopic and histopathological examination and fetal blood culture (including screening for pathogenic bacteria associated with stillbirth). Cases missing one or more of these investigations were considered to have incomplete samples and were excluded from the analysis of cause of stillbirth. Causes of stillbirths were assessed by individual case reviews by at least two obstetricians, and classified with a modified Stillbirth Collaborative Research Network classification system.

Findings Between Oct 9, 2014, and Nov 8, 2015, we enrolled 354 stillbirths (born to 350 women). Among the women with available data, 133 (38%) of 350 had hypertension, median age was 27 years (IQR 23–33), 51 (18%) of 291 were obese, six (2%) of 344 had syphilis, and 94 (27%) of 350 had HIV. 63 (18%) of 341 fetuses showed intrauterine growth restriction. Of 298 cases (born to 294 mothers) with complete samples, the most common causes of stillbirth were maternal medical conditions (64 [21%] cases; among them 56 [19%] with hypertensive disorders and six [2%] with diabetes), placental or fetal infections (58 [19%]; 47 [16%] with fetal invasive bacterial infection), pathological placental conditions (57 [19%]; among them 27 [9%] with fetal membrane and placental inflammation and 26 [9%] with circulatory abnormalities), and clinical obstetric complications (54 [18%]; 45 [15%] with placental abruption). Six (2%) stillbirths were attributed to fetal, genetic, or structural abnormalities. In 55 (18%) cases, no cause of death was identified. The most common bacteria to which stillbirths due to fetal invasive infections were attributed were group B streptococcus (15 [5%] cases), *E coli* (12 [4%]), *E faecalis* (six [2%]), and *S aureus* (five [2%]).

Interpretation Targeted investigation of stillbirths (even without fetal autopsy) can ascertain a cause of stillbirth in most cases. Further studies using such investigations are needed to inform the prioritisation of interventions to reduce stillbirths globally.

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Introduction

Stillbirths are associated with immense socioeconomic consequences, including impaired physical and mental wellbeing of bereaved parents and financial costs to families and the health-care system.¹ In 2014, the need to reduce stillbirths in low-income and middle-income countries (LMICs) was acknowledged in the World Health Assembly-endorsed Every Newborn Action Plan, which targets reducing the number of stillbirths per 1000 births from 18·4 in 2015 to 12 by 2030.² In 2015, there were 2·6 million third-trimester stillbirths, of which 41% occurred in African LMICs and 36% occurred in south Asian LMICs.^{3,4} However, despite this burden being almost equal to the number of neonatal deaths (2·7 million) that occurred in 2015,⁵ biological investigations of the causes of stillbirths in LMICs are scarce.

A systematic review of the causes of stillbirths in LMICs and high-income countries between 2009 and 2016 highlighted the poor quality of data,⁶ which is recognised as a major gap for determining strategies aimed at reducing stillbirths.² The use of different classification systems (n=34), and heavy reliance on retrospectively collated administrative data or verbal autopsies in LMICs for attributing the causes of death, further undermine our understanding of the epidemiology of stillbirths. Only seven of 37 studies from high-income countries and none from LMICs used a comprehensive systematic protocol for investigating stillbirths. Furthermore, placental examination, which is considered highly informative in attributing stillbirth causes,^{7,8} was not done in any of 28 studies from low-income countries, and in only a small number of cases in two studies in middle-income settings.⁶ Overall, 32% of

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Research in context

Evidence before this study

Current understanding on the causes of stillbirths in low-income and middle-income countries (LMICs) is largely premised on the use of vital registration data (which is estimated to record less than 5% of all stillbirths), and verbal autopsy data that has not been validated against autopsies or biological investigation of the fetus and placenta. A 2018 systematic review of stillbirths between 2009 and 2016 highlighted the poor quality of data, inconsistent use of classification systems, and reliance on administrative data and verbal autopsies for attributing cause of fetal death. Although placental examination is highly informative for attributing causes of death, none of the studies from low-income settings, and only two small studies from middle-income settings included investigation of the placenta when studying the causes of stillbirth. The meta-analysis concluded that 41–44% of stillbirths in LMICs were unexplained, and the leading causes in low-income countries were infection (15.8%), hypoxic peripartum death (11.6%), antepartum haemorrhage (9.3%), and other unspecified conditions (13.8%). Another study investigated 2847 stillbirths enrolled between 2014 and 2015 in seven low-income countries with use of a computer-based hierarchical algorithm to assign causes of stillbirth on the basis of a medical review of conditions present during pregnancy and delivery. This study also did not undertake any biological investigation of the fetus or placenta. In this study, stillbirths were attributed to asphyxia (46.6%), infection (20.8%), congenital anomalies (8.4%), and prematurity (6.6%), and no cause of death was identified in 11.8% of cases. Notably, because of the absence of biological investigation of stillbirths in the aforementioned multicentre study and in most studies included in the meta-analysis, the specificity of diagnosis and causes of infections were not ascertainable. Similarly, the underlying causes of the asphyxia were not fully characterised in the absence of placental investigation.

Added value of this study

In this prospective, observational study done in South Africa, in addition to review of available maternal medical records

during pregnancy and labour, we undertook macroscopic and histological examination of the placenta and sent fetal blood for culture. Obstetricians arbitrated on the causes of stillbirth and categorised these causes with a modified Stillbirth Collaborative Research Network classification system. With this approach, we identified a possible or probable underlying maternal, placental, or fetal cause of stillbirth in 82% of cases. Underlying maternal medical conditions (64 [21%] of 298 cases; mainly hypertension, in 56 [19%] cases); infections of the placenta or fetus (58 [19%]), pathological placental conditions (57 [19%], including 27 [9%] with fetal membrane and placental inflammation and 26 [9%] with circulatory abnormalities); and clinical obstetric complications (54 [18%]) contributed to a similar proportion of fetal deaths. We also characterised the specific pathogens responsible for fetal invasive bacterial infections, which included Group B streptococcus, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Furthermore, although 94 (27%) of 350 screened women were positive for HIV and six (2%) were positive for syphilis, none of the stillbirths in these cases were recorded as being caused by these infections.

Implications of all the available evidence

This study highlights the need for systematic investigation of stillbirths—which, in addition to medical record review, should include at least fetal blood culture and macroscopic and microscopic investigation of the placenta—in low-income and middle-income settings. The identification of the specific underlying maternal and fetal conditions resulting in stillbirth is fundamental to ascertaining which tools should be scaled up, especially for stillbirths that are not due to intrapartum obstetric complications (which itself should be addressed). Further studies like this one, and which include tests for a broader range of infections (such as possible viral causes of stillbirth), are warranted in other settings with a high incidence of stillbirths to help meet the goals of the Every Newborn Action Plan by 2030.

stillbirths from high-income countries and 41–44% from LMICs were unexplained in the meta-analysis.⁶ In addition, although 16% of stillbirths in low-income countries were attributed to infection, this attribution was mainly based on verbal autopsy or retrospective hospital audits, without pathogen-specific causes identified.

The aim of this prospective, observational study was to investigate the causes of stillbirths among South African women, including a focus on the role of fetal invasive bacterial infection.

Methods

Study design and setting

We enrolled women who had stillbirths at the Chris Hani-Baragwanath Academic Hospital, a public hospital in Soweto, Johannesburg, South Africa, between

Oct 9, 2014, and Nov 8, 2015. The study setting favours comprehensive capture of stillbirths occurring in Soweto, as detailed in the appendix. Based on administrative databases from Soweto, there were 22.5 stillbirths per 1000 births in 2015 (figure).

The study was approved by the Human Research Ethics Committee at University of the Witwatersrand (number 131008) and registered with ClinicalTrials.gov (number NCT02339077). Written, informed consent was obtained from each mother before sample or data collection.

Study procedures

Study staff were stationed in the labour ward to enrol consenting women aged 18 years or older who had a stillbirth (defined as an infant with no signs of life at 1 min

See Online for appendix

and 5 min [APGAR 0]). We limited enrolment to fetuses of at least 22 weeks' gestational age or with a birthweight of at least 500 g. Gestational age staging was based on available obstetric assessment using ultrasound, last menstrual period date, or serial antenatal symphysis-fundal height measurements. Women who had stillbirths that had already been delivered at a midwife-operated unit and who were referred to Chris Hani-Baragwanath Academic Hospital were not approached for enrolment because of the unavailability of the placenta in these cases (figure).

Maternal and fetal clinical information was abstracted after enrolment of the case from the medical records of the attending health workers. This information was collected by study nurses and reviewed by a medical doctor (CB) using a standard data-abstraction form, and included antenatal syphilis and HIV serology test results and related treatment. Mid-upper arm circumference was measured as a proxy for the nutritional status of the mother (<23 cm indicating malnourishment and >33 cm indicating obesity).

Laboratory procedures

After delivery, the placenta was retrieved and weighed, and a wedge of placental parenchyma and membranes resected and placed in a sterile container for bacterial microscopy and culture. The remaining placenta, membranes, and cord were immersed in 10% buffered formalin and transported to Lancet Laboratories (Johannesburg, South Africa), where they were weighed, measured, and examined macroscopically. A histopathologist selected portions of placenta, which were embedded in paraffin and processed for routine haematoxylin and eosin staining with standard protocols.⁹ Placental assessment by the histopathologist was based on the Stillbirth Collaborative Research Network (SCRN) protocol for placental and umbilical cord examination.¹⁰

Sampling from stillbirths included blood collection. We initially attempted to collect cord blood; however, technical challenges in very premature or macerated fetuses required amendment of the blood collection procedure to include obtaining fetal blood by cardiac puncture if cord-blood sampling failed. Cord or skin surfaces were decontaminated with alcohol solution before blood sampling, and blood (0.5–5.0 mL) was inoculated into BacT/ALERT PF Plus culture bottle and assessed with the BacT/ALERT 3D microbial detection system (BioMerieux, Marcy l'Etoile, France). Positive cultures from all samples were Gram-stained and underwent identification and susceptibility testing, mainly by use of the Kirby-Bauer manual method. Syphilis screening of stillbirth blood was done at the National Health Laboratory Service at Chris Hani-Baragwanath Academic Hospital with use of the *Treponema pallidum* antibody test (Architect Syphilis TP assay; Abbott, Wiesbaden, Germany), and positive samples were assessed for disease activity and treatment

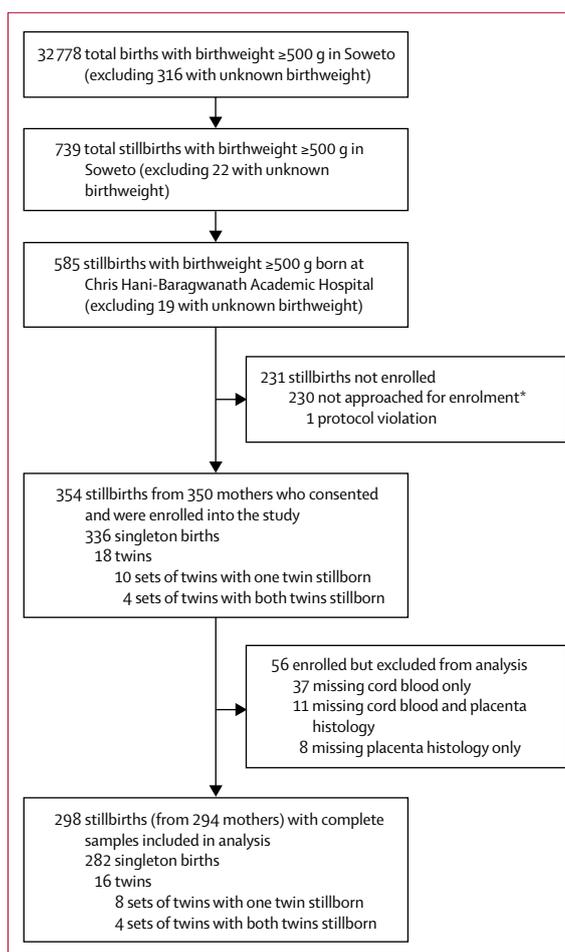


Figure: Stillbirths in Soweto between Oct 9, 2014, and Nov 8, 2015

*These cases were not approached for study participation because enrolment was limited to 1–2 cases per day because of logistical constraints.

status with rapid plasma reagin. An aliquot of serum was archived at -70°C .

Individual cases were reviewed by at least two obstetricians (SMa, SMO, or PM), and stillbirth causes were classified with a modified SCRN classification system (appendix).¹¹ If the initial panellists did not concur on the cause of a stillbirth, the case was adjudicated by another medical doctor (RC) or obstetrician (SMa, SMO, PM, or YA). The SCRN classification records conditions as being present but not necessarily contributory to the stillbirth, or as a possible or probable cause of death, with varying levels of evidence (appendix). Prevailing medical conditions potentially related to the stillbirth were ascertained, including multiple conditions per individual case (appendix). The final cause of stillbirth was, however, limited to a single cause graded as possibly or probably related. In cases with multiple possible or probable associated conditions, the condition in the absence of which the stillbirth would not have occurred or which precipitated the sequence of events that led to the stillbirth was identified as the main underlying cause (appendix).

Bacteria previously reported¹² as possible causes of stillbirth were evaluated for causality in the enrolled cases, including growth from fetal blood of *Escherichia coli*, *Enterobacter* sp, *Enterococcus faecalis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and group B streptococcus (GBS). Although greater weight was placed on pure growth of these bacteria on culture, cases in which a potential contaminant was concurrently cultured were also considered. Although placental tissue

was also cultured for bacteria, these results were interpreted with circumspection because of the high possibility of microbial contamination.

Statistical analysis

The study sample size was premised on the assumption that as many as 50% of stillbirths in developing countries could be due to infections, and we targeted enrolling 300 cases to provide 80% power to identify GBS as a

	Total (n=350)	Complete sampling (n=294)	Incomplete sampling (n=56)	p value*
Age, years	27 (23–33)	27 (23–33)	27 (25–31)	0.87
≥35	59/344 (17%)	52/289 (18%)	7/55 (13%)	0.44
<35	285/344 (83%)	237/289 (82%)	48/55 (87%)	..
MUAC, cm	29.4 (4.5)	29.4 (4.6)	29.6 (4.2)	0.79
>33 (obese)	51/291 (18%)	42/248 (17%)	9/43 (21%)	0.52
<23 (malnourished)	11/291 (4%)	10/248 (4%)	1/43 (2%)	>0.99
Parity (before current pregnancy)	1 (1–2)	1 (1–2)	1 (1–2)	0.35
0	44/253 (17%)	40/212 (19%)	4/41 (10%)	0.12
1–4	204/253 (81%)	169/212 (80%)	35/41 (85%)	..
≥5	5/253 (2%)	3/212 (1%)	2/41 (5%)	..
At least one antenatal care visit	322/350 (92%)	271/294 (92%)	51/56 (91%)	0.79
Multiple gestation (twins)	14/350 (4%)	12/294 (4%)	2/56 (4%)	>0.99
Previous stillbirth	26/350 (7%)	19/294 (6%)	7/56 (13%)	0.091
Timing of stillbirth	0.45
Antepartum	227/350 (65%)	188/294 (64%)†	39/56 (70%)	..
Intrapartum	123/350 (35%)	106/294 (36%)‡	17/56 (30%)	..
Diagnosis of stillbirth	>0.99
After delivery	54/344 (16%)	46/289 (16%)	8/55 (15%)	..
Predelivery ultrasound	290/344 (84%)	243/289 (84%)	47/55 (85%)	..
Mode of delivery	0.87
Vaginal	259/350 (74%)	218/294 (74%)	41/56 (73%)	..
Caesarean section	91/350 (26%)	76/294 (26%)	15/56 (27%)	..
HIV infected	94/350 (27%)	82/294 (28%)	12/56 (21%)	0.41
Receiving antiretroviral therapy for HIV	82/94 (87%)	75/82 (91%)	7/12 (58%)	0.0070
Duration of antiretroviral therapy, months§	2 (1–4)	2 (1–4)	3 (3–4)	0.37
Tobacco smoker¶	6/195 (3%)	4/163 (2%)	2/32 (6%)	0.26
Alcohol use during pregnancy¶¶	9/190 (5%)	6/159 (4%)	3/31 (10%)	0.17
Maternal syphilis				
Tested	344/350 (98%)	292/294 (99%)	52/56 (93%)	0.0069
Positive	6/344 (2%)	6/292 (2%)	0/52	0.60
Pregnancy-induced hypertension	111/350 (32%)	93/294 (32%)	18/56 (32%)	>0.99
Chronic hypertensive disorder	22/350 (6%)	18/294 (6%)	4/56 (7%)	0.76
Pregnancy-associated diabetes	8/350 (2%)	7/294 (2%)	1/56 (2%)	>0.99
Other chronic medical conditions	2/350 (1%)	2/294 (1%)	0/56	>0.99
Intrapartum complications				
Cord prolapse	4/350 (1%)	4/294 (1%)	0/56	>0.99
Prolonged labour**	31/129 (24%)	25/107 (23%)	6/22 (27%)	0.78
Fetal distress	34/350 (10%)	30/294 (10%)	4/56 (7%)	0.63
Placental abnormalities (clinically diagnosed)				
Placenta praevia	2/350 (1%)	1/294 (<1%)	1/56 (2%)	0.30
Placental abruption††	67/350 (19%)	62/294 (21%)	5/56 (9%)	0.040
Uterine rupture	4/350 (1%)	4/294 (1%)	0/56	>0.99

(Table 1 continues on next page)

	Total (n=350)	Complete sampling (n=294)	Incomplete sampling (n=56)	p value*
(Continued from previous page)				
Evidence of maternal infection during pregnancy or labour	27/350 (8%)	23/294 (8%)	4/56 (7%)	>0.99
Intrapartum temperature $\geq 38^{\circ}\text{C}$	4/350 (1%)	4/294 (1%)	0/56	>0.99
Clinically diagnosed chorioamnionitis	3/350 (1%)	2/294 (1%)	1/56 (2%)	0.41
Urinary tract infection	14/350 (4%)	11/294 (4%)	3/56 (5%)	0.48
Malodorous or purulent vaginal discharge	8/350 (2%)	7/294 (2%)	1/56 (2%)	>0.99
Uterine tenderness	1/350 (<1%)	1/294 (<1%)	0/56	>0.99

Data are n/N (%), mean (SD), or median (IQR). MUAC=mid-upper arm circumference. *Complete versus incomplete sampling groups. †Delivered 190 stillbirths. ‡Delivered 108 stillbirths. §From time of initiation to time of birth in HIV-infected women. ¶Quantity not ascertainable from medical records. ||Includes one mother with cardiac abnormality and one with thyroid dysfunction. **Defined as ≥ 20 h in nulliparous and ≥ 14 h in multiparous women. ††Overall 38 (13%) of 290 stillbirths diagnosed predelivery on ultrasound, including 37 (15%) of 243 with complete sampling, and 1 (2%) of 47 with incomplete sampling (p=0.014).

Table 1: Demographic and baseline clinical features of women who had stillbirths

cause of such infections in up to 10% of infection-related stillbirths.

Analyses were limited to woman–stillbirth dyads that had available medical clinical records, fetal blood culture, and placental macroscopic and histological results. Z scores and percentiles for fetal growth retardation were calculated with the INTERGROWTH-21st standards.¹³ For stillbirths with a gestational age of 24–43 weeks, growth standards for preterm and term newborns were applied. Fetal growth standards were applied to stillbirths of less than 24 weeks' gestational age.

Maternal and fetal demographic and clinical features are reported as frequency (with %), mean (with SD), or median (with IQR). Categorical variables were compared with the Fisher's exact test. A Wilcoxon rank-sum test was used to compare medians, and a two-sample t test was used to compare means between two groups. All statistical analyses were done with R version 3.4.

Role of the funding source

The funding source played no role in the study design, data collection and analyses, preparation or approval of the manuscript. The funder was provided the opportunity to review a preliminary version of this manuscript for factual accuracy, but the authors were solely responsible for final content and interpretation.

Results

Of the 354 stillbirths (350 mothers) enrolled between Oct 9, 2014, and Nov 8, 2015, complete samples were available for 298 (84%) stillbirths born to 294 women (figure). The main reason for missing data was unavailability of blood for culture (37 of 56 cases), which preceded allowing for blood to be collected by cardiac puncture if cord-blood sampling failed. There were higher proportions of HIV-infected women receiving antiretroviral treatment, women tested for syphilis, and diagnoses of placental abruption among woman–stillbirth dyads with complete study data than among those without (table 1). Among the stillbirths, those with

	Total (n=354)	Complete sampling (n=298)	Incomplete sampling (n=56)	p value*
Sex	0.15
Male	190/354 (54%)	165/298 (55%)	25/56 (45%)	..
Female	164/354 (46%)	133/298 (45%)	31/56 (55%)	..
Birthweight, g	1700 (940–2461)	1812 (1006–2531)	1020 (735–1760)	<0.00032
500–999	98/347 (28%)	72/294 (24%)	26/53 (49%)	0.0018
1000–1499	55/347 (16%)	45/294 (15%)	10/53 (19%)	..
1500–2499	109/347 (31%)	100/294 (34%)	9/53 (17%)	..
2500–4000	80/347 (23%)	73/294 (25%)	7/53 (13%)	..
>4000	5/347 (1%)	4/294 (1%)	1/53 (2%)	..
Gestational age, weeks	33 (27–37)	33 (28–37)	29 (26–36)	0.018
22–27	88/347 (25%)	68/293 (23%)	20/54 (37%)	0.0497
28–32	85/347 (24%)	68/293 (23%)	17/54 (31%)	..
33–36	73/347 (21%)	67/293 (23%)	6/54 (11%)	..
37–41	91/347 (26%)	81/293 (28%)	10/54 (19%)	..
≥ 42	10/347 (3%)	9/293 (3%)	1/54 (2%)	..
Fetal growth retardation				
Z score† ≤ -2	63/341 (18%)	52/290 (18%)	11/51 (22%)	0.56
Z score ≤ -3	15/341 (4%)	12/290 (4%)	3/51 (6%)	0.48
<10th percentile	106/341 (31%)	87/290 (30%)	19/51 (37%)	0.33
Fetal appearance	0.086
Macerated	242/353 (69%)	198/297 (67%)	44/56 (79%)	..
Fresh	111/353 (31%)	99/297 (33%)	12/56 (21%)	..
Congenital abnormalities	16/354 (5%)	13/298 (4%)‡	3/56 (5%)§	0.73

Data are n/N (%) or median (IQR). *Complete versus incomplete sampling groups. †Z score for birthweight for gestational age. ‡Comprising anencephaly (n=1), hydrups fetalis (n=3), exomphalos (n=2), Arnold-Chiari syndrome with hydrocephalus (n=1), other brain malformation (n=2), congenital malformation of face and neck, unspecified (n=1), congenital malformation of musculoskeletal system (n=1), malformation of heart (n=1), and trisomy 18 (n=1). §Comprising unspecified congenital hydrocephalus (n=1), congenital malformations of heart (n=1), and congenital malformation of musculoskeletal system (n=1).

Table 2: Baseline demographic and clinical features of the stillbirths

complete sampling were of higher median weight and gestational age (table 2). Subsequent analyses were limited to the woman–stillbirth dyads with complete samples.

Among cases with complete samples, most stillbirths (243 [84%] of 289) were diagnosed predelivery by

	Overall (n=298)*	Antepartum stillbirths (n=190)	Intrapartum stillbirths (n=108)	p value†
Umbilical cord				
Cord insertion	0.72
Central	131/262 (50%)	84/172 (49%)	47/90 (52%)	..
Eccentric	122/262 (47%)	82/172 (48%)	40/90 (44%)	..
Marginal	7/262 (3%)	5/172 (3%)	2/90 (2%)	..
Interpositional	1/262 (<1%)	1/172 (1%)	0/90	..
Velamentous	1/262 (<1%)	0/172	1/90 (1%)	..
Number of cord vessels: 3	288/288 (100%)	188/188 (100%)	100/100 (100%)	>0.99
Cord thrombosis	6/298 (2%)	0/190	6/108 (6%)	0.0021
Cord knots	1/298 (<1%)	1/190 (1%)	0/108	>0.99
Funisitis	6/298 (2%)	2/190 (1%)	4/108 (4%)	0.19
Placental membranes				
Membrane colour	0.20
Translucent	213/266 (80%)	140/171 (82%)	73/95 (77%)	..
Green	7/266 (3%)	5/171 (3%)	2/95 (2%)	..
Yellow or brown	5/266 (2%)	1/171 (1%)	4/95 (4%)	..
Other	41/266 (15%)	25/171 (15%)	16/95 (17%)	..
Amnion nodosum	4/296 (1%)	2/189 (1%)	2/107 (2%)	0.62
Chorioamnionitis	75/296 (25%)	42/189 (22%)	33/107 (31%)	0.13
Chorionic vasculitis	8/298 (3%)	7/190 (4%)	1/108 (1%)	0.27
Placental parenchyma				
Macroscopic infarct	90/296 (30%)	64/189 (34%)	26/107 (24%)	0.089
Marked calcification	23/296 (8%)	18/189 (10%)	5/107 (5%)	0.18
Colour	0.048
Normal	81/296 (27%)	51/189 (27%)	30/107 (28%)	..
Pale	108/296 (36%)	78/189 (41%)	30/107 (28%)	..
Congested	107/296 (36%)	60/189 (32%)	47/107 (44%)	..
Retroplacental haematoma	60/296 (20%)	40/189 (21%)	20/107 (19%)	0.65
Placental infarction‡	0.40
Yes	149/296 (50%)	99/189 (52%)	50/107 (47%)	..
<10%	18/131 (14%)	13/89 (15%)	5/42 (12%)	0.12
10–20%	61/131 (47%)	35/89 (39%)	26/42 (62%)	..
21–30%	31/131 (24%)	24/89 (27%)	7/42 (17%)	..
>30%	21/131 (16%)	17/89 (19%)	4/42 (10%)	..
Intervillous thrombi	1/296 (<1%)	1/189 (1%)	0/107	>0.99
Intervillous fibrin	125/296 (42%)	78/189 (41%)	47/107 (44%)	0.71
Villitis	2/296 (1%)	1/189 (1%)	1/107 (1%)	>0.99

Data are n/N (%). *Includes four sets of twins from which both twins were assessed, including two sets with a shared placenta and two sets with separate placentas. †Comparison of antepartum and intrapartum cases. ‡Observations on dissected placental tissue.

Table 3: Macroscopic and histological placental observations in stillbirths

ultrasonography, and only 46 (16%) were diagnosed at delivery. On the basis of ultrasonography or clinical examination, 188 (64%) of 294 deaths with available data were categorised as antepartum and 106 (36%) as intrapartum. 79 (27%) of 298 stillbirths were delivered by caesarean section (table 1), of which 69 (87%) were for a medical emergency indication. The median age of mothers at the time of birth was 27 years (IQR 23–33), with 52 (18%) aged 35 years or older. The mean mid-upper arm circumference among 248 women with available data was 29.4 cm (SD 4.6), with 42 (17%) women

categorised as obese and ten (4%) as malnourished. Most women had at least one antenatal care visit before delivery. 82 (28%) of 294 women with available data were HIV-infected (75 [91%] of whom were on antiretroviral treatment before delivery, for a median of 2 months [IQR 1–4]). Six (2%) of 350 mothers were seropositive for syphilis. Other known maternal risk factors for stillbirths⁴ included pregnancy-induced hypertension, chronic hypertension, and pregnancy-associated diabetes. Among those for whom data were available, tobacco smoking and alcohol use during pregnancy were uncommon (table 1). Intrapartum complications included prolonged labour, clinically diagnosed placental abruption, and uterine rupture. Furthermore, 27 (8%) women had clinically diagnosed infections immediately before or at onset of labour (table 1). Two maternal deaths occurred: one due to diabetes ketoacidosis and one due to eclampsia.

Among stillbirths with complete samples available, slightly more than half were male, median birthweight was 1812 g (IQR 1006–2531), and median gestational age was 33 weeks (28–37; table 2). Around three-quarters of stillbirths weighed at least 1000 g at birth and a similar proportion were of at least 28 weeks' gestational age. 52 (18%) of 290 stillbirths with available data had intrauterine growth restriction (weight for gestational age Z score less than –2). Around two-thirds of stillbirths had a macerated appearance, including 149 (78%) of 190 antepartum and 49 (45%) of 108 intrapartum stillbirths (p<0.001 for antepartum vs intrapartum). Clinically evident congenital abnormalities were present in 13 (4%) of 298 stillbirths with complete samples (table 2).

Cord thrombosis was more common among intrapartum (6 [6%] of 108) than antepartum cases (0 of 190; p=0.0021). Other cord abnormalities observed included funisitis and cord knots (table 3). Chorioamnionitis was histologically confirmed in 75 (25%) of 296 placentas, with 72 (96%) of these categorised as acute. Information on the extent of placental inflammatory cellular infiltrate was available for 59 (79%) of the placentas with chorioamnionitis, and was assessed as grade II in 25 (42%) and grade III in 7 (12%) cases. Macroscopic infarcts were found in 90 (30%) placentas (including 64 [34%] antepartum and 26 [24%] intrapartum cases; p=0.089), and 23 (8%) placentas had marked calcification. Upon placental dissection, infarcts were evident in 149 (50%) placentas, with 52 (40%) of 131 involving more than 20% of the cut surface. Infarcts involving more than 20% of the placental cut surface were more common among antepartum than intrapartum cases (table 3). Most (72 [91%] of 79) infarcts were classified as old.

Potentially pathogenic bacterial species or genera previously associated with stillbirths¹² were cultured from fetal blood in 81 (27%) cases, including 44 (15%) cases in which the bacteria were cultured in the absence of a concurrent probable contaminant (eg, *Bacillus* sp or *Staphylococcus epidermidis*). Seven potentially pathogenic

species of bacteria were found in at least one case each, the most common being *E faecalis* (table 4). The growth of a potential pathogen was slightly less common among antepartum than intrapartum cases, although this difference was not significant (table 4).

Bacteria potentially associated with stillbirths were cultured from 179 (62%) of 289 placental tissues, and in pure culture from 61 (21%) placental tissues (appendix). The most common bacteria identified in the absence of a probable contaminant were *E coli* (31 cases [11%]), *E faecalis* (29 [10%]), and group B streptococcus (21 [7%]).

The overall prevalence of medical conditions, irrespective of whether they were associated with causing the stillbirth and inclusive of multiple diagnoses per case, is detailed in the appendix. In 243 (82%) of 298 cases, the possible or probable cause of stillbirth was attributed to a single underlying maternal or fetal condition, whereas no cause was evident for 55 (18%) cases (table 5, appendix). Overall, deaths were most commonly attributed to maternal medical conditions (64 [21%] cases), infections of the placenta or fetus (58 [19%]), pathological placental conditions (57 [19%]), and clinical obstetric complications (54 [18%]; table 5). Only six (2%) stillbirths were attributed to fetal abnormalities, including one (<1%) case of hydrocephalus, one (<1%) case of extensive intracerebral bleeding, and one (<1%) case of anencephaly in which the pregnancy was medically terminated.

Of the maternal medical conditions to which stillbirths were attributed, the most common were hypertension (56 [19%] cases) and diabetes (six [2%]; table 5, appendix). Among the clinical obstetric conditions, placental abruption was most common (45 [15%]), and five (2%) stillbirths were attributed to uterine rupture. The main underlying placental abnormalities recorded as the cause of stillbirth were inflammation of the fetal membranes and placenta (27 [9%]) and placental circulatory abnormalities (eg, infarcts; 26 [9%]). Underlying causes did not differ significantly in frequency between antepartum and intrapartum stillbirths (table 5).

Among stillbirths attributed to infection, 47 (16%) were due to fetal invasive bacterial infection and 11 (4%) to placental bacterial infection (table 5, appendix). The most common bacteria recorded as causing fetal invasive infections that led to stillbirth were group B streptococcus (15 [5%] cases), *E coli* (12 [4%]), *E faecalis* (six [2%]), and *S aureus* (five [2%]). These frequencies were lower than the overall frequencies of fetal invasive bacterial infections (irrespective of whether they were the cause of stillbirth), with *E coli* found in 40 cases (13%), group B streptococcus in 24 (8%), *Pseudomonas aeruginosa* in 14 (5%), *S aureus* in 13 (4%), and *E faecalis* in seven (2%; appendix).

Discussion

In this prospective, observational study in a low-to-middle income African setting, we used a systematic

	Overall (n=298)	Antepartum stillbirths (n=190)	Intrapartum stillbirths (n=108)	p value*
Potential pathogen				
With or without contaminant	81 (27%)	46 (24%)	35 (32%)	0.138
Pure culture only	44 (15%)	27 (14%)	17 (16%)	0.736
<i>Enterococcus faecalis</i>				
With or without contaminant	41 (14%)	25 (13%)	16 (15%)	0.728
Pure culture only	23 (8%)	17 (9%)	6 (6%)	0.369
<i>Escherichia coli</i>				
With or without contaminant	31 (10%)	16 (8%)	15 (14%)	0.167
Pure culture only	15 (5%)	9 (5%)	6 (6%)	0.787
Group B streptococcus				
With or without contaminant	17 (6%)	7 (4%)	10 (9%)	0.067
Pure culture only	12 (4%)	5 (3%)	7 (6%)	0.128
<i>Klebsiella pneumoniae</i>				
With or without contaminant	12 (4%)	8 (4%)	4 (4%)	>0.999
Pure culture only	5 (2%)	4 (2%)	1 (1%)	0.657
<i>Staphylococcus aureus</i>				
With or without contaminant	10 (3%)	6 (3%)	4 (4%)	0.752
Pure culture only	7 (2%)	6 (3%)	1 (1%)	0.428
<i>Enterobacter</i> sp				
With or without contaminant	6 (2%)	4 (2%)	2 (2%)	>0.999
Pure culture only	2 (1%)	1 (1%)	1 (1%)	>0.999
<i>Enterococcus faecium</i>				
With or without contaminant	4 (1%)	1 (1%)	3 (3%)	0.137
Pure culture only	3 (1%)	1 (1%)	2 (2%)	0.298
Negative on culture	109 (37%)	69 (36%)	40 (37%)	0.901
Contaminant only identified	107 (36%)	74 (39%)	33 (31%)	0.168

Data are n (%). *Antepartum versus intrapartum cases.

Table 4: Pathogenic bacteria cultured from cord or heart blood samples from stillbirths

approach to investigate and categorise stillbirths and identified a possible or probable underlying cause in 243 (82%) of 298 cases. Most of these causes were potentially preventable or treatable, or could have interventions developed. Included among these causes were probable fetal compromise (ie, intrauterine asphyxia) due to underlying maternal hypertensive disorders (19%) and antepartum haemorrhage (17%; 15% due to placental abruption and 2% due to uterine rupture). Furthermore, 19% of stillbirths were attributed to pathological placental conditions, including 9% due to inflammation of the fetal membranes and placenta (ie, chorioamnionitis).

Another major underlying cause of stillbirths was fetal invasive bacterial infection, which was recorded as possibly or probably the main cause of death in around 16% of cases. Bacteria causing invasive fetal infection in our study included group B streptococcus, *E coli*, *E faecalis*, and *S aureus*, species which are also commonly associated with rectovaginal tract colonisation in pregnant women in Soweto.¹⁴ Although our observation of the prevalence of infection as cause of stillbirth is similar to that estimated for low-income countries in a systematic review (15.8%)⁶ and another multicentre study in seven LMICs (20%),¹⁵

	Overall			Antepartum stillbirths			Intrapartum stillbirths			p value*
	Total (n=298)	Probable (n=201)	Possible (n=42)	Total (n=190)	Probable (n=134)	Possible (n=25)	Total (n=108)	Probable (n=67)	Possible (n=17)	
Maternal medical condition during pregnancy	64 (21%)	57 (28%)	7 (17%)	46 (24%)	42 (31%)	4 (16%)	18 (17%)	15 (22%)	3 (18%)	0.14
Hypertensive disorder	56 (19%)	52 (26%)	4 (10%)	40 (21%)	38 (28%)	2 (8%)	16 (15%)	14 (21%)	2 (12%)	..
Diabetes	6 (2%)	4 (2%)	2 (5%)	4 (2%)	3 (2%)	1 (4%)	2 (2%)	1 (1%)	1 (6%)	..
Other	2 (1%)	1 (<1%)	1 (2%)	2 (1%)	1 (1%)	1 (4%)	0	0	0	..
Clinical obstetric complications	54 (18%)	53 (26%)	1 (2%)	32 (17%)	32 (24%)	0	22 (20%)	21 (31%)	1 (6%)	0.53
Clinical chorioamnionitis	1 (<1%)	1 (<1%)	0	1 (1%)	1 (1%)	0	0	0	0	..
Intrapartum fetal distress with asphyxia or hypoxic intrapartum fetal distress	2 (1%)	2 (1%)	0	1 (1%)	1 (1%)	0	1 (1%)	1 (1%)	0	..
Placental abruption	45 (15%)	44 (22%)	1 (2%)	26 (14%)	26 (19%)	0	19 (18%)	18 (27%)	1 (6%)	..
Uterine rupture	5 (2%)	5 (2%)	0	3 (2%)	3 (2%)	0	2 (2%)	2 (3%)	0	..
Uteroplacental insufficiency	1 (<1%)	1 (<1%)	0	1 (1%)	1 (1%)	0	0	0	0	..
Fetal, genetic, or structural abnormality	6 (2%)	5 (2%)	1 (2%)	5 (3%)	4 (3%)	1 (4%)	1 (1%)	1 (1%)	0	0.42
Infection	58 (19%)	40 (20%)	18 (43%)	37 (19%)	26 (19%)	11 (44%)	21 (19%)	14 (21%)	7 (41%)	>0.99
Placental infection and decreased placental function	11 (4%)	6 (3%)	5 (12%)	7 (4%)	3 (2%)	4 (16%)	4 (4%)	3 (4%)	1 (6%)	..
Fetal bacterial infection	47 (16%)	34 (17%)	13 (31%)	30 (16%)	23 (17%)	7 (28%)	17 (16%)	11 (16%)	6 (35%)	..
Pathological placental conditions	57 (19%)	43 (21%)	14 (33%)	37 (19%)	28 (21%)	9 (36%)	20 (19%)	15 (22%)	5 (29%)	0.88
Placental disc	1 (<1%)	1 (<1%)	0	1 (1%)	1 (1%)	0	0	0	0	..
Placental membranes	1 (<1%)	1 (<1%)	0	1 (1%)	1 (1%)	0	0	0	0	..
Umbilical cord	1 (<1%)	0	1 (2%)	1 (1%)	0	1 (4%)	0	0	0	..
Fetal membrane and placental inflammation	27 (9%)	22 (11%)	5 (12%)	13 (7%)	12 (9%)	1 (4%)	14 (13%)	10 (15%)	4 (24%)	..
Circulatory abnormalities	26 (9%)	19 (9%)	7 (17%)	20 (11%)	14 (10%)	6 (24%)	6 (6%)	5 (7%)	1 (6%)	..
Other placenta abnormalities	1 (<1%)	0	1 (2%)	1 (1%)	0	1 (4%)	0	0	0	..
Other	4 (1%)	3 (1%)	1 (2%)	2 (1%)	2 (1%)	0	2 (2%)	1 (1%)	1 (6%)	0.62
Unknown	55 (18%)	0	0	31 (16%)	0	0	24 (22%)	15 (22%)	0	0.22

Data are n (%) for cases with complete samples. Details of the specific medical conditions and criteria upon which the diagnoses were based are reported in the appendix. The criteria used for categorisation of diagnoses as probable or possible are based on the Modified Stillbirth Collaborative Research Network classification system¹² (appendix). *Comparison of totals for antepartum and intrapartum stillbirth causes. All sub-analysis p values were >0.05.

Table 5: Main maternal or fetal conditions that possibly or probably contributed to the stillbirth

those estimates were largely premised on the prevalence of maternal or fetal malaria and syphilis infections, or on fetal or vaginal odour as proxies for bacterial vaginosis. On the basis of clinical history, 55.7% of stillbirths in the Democratic Republic of the Congo, 34.7% in Kenya, and 17.9% in Zambia were attributed to infections.¹⁵ Nevertheless, since causes of stillbirth in LMICs are largely based on verbal autopsy, retrospective record reviews, or vital registration data,^{6,15} there is a paucity of data on which organisms are involved in the pathogenesis of stillbirths related to invasive bacterial infections, and there could be a general under-recognition of the role of these infections. For example, global estimates of the causes of stillbirths have been modelled with use of available data on the prevalence of recognised risk factors and related risk for stillbirths, on the basis of which 7.7% of stillbirths in LMICs were attributed to syphilis, 8.0% to malaria, and 0.7% to HIV, whereas invasive fetal bacterial infection was not listed as a cause.⁴

In our study, which was set in a malaria non-endemic area, no stillbirths were attributed to malaria. Furthermore, although six (2%) women were seropositive for syphilis, stillbirths were not attributed to syphilis in any of these cases, but rather to other conditions (three fetal infections, one placental infection, one due to maternal diabetes, and one due to an unknown cause). This finding probably reflects the high proportion of women who attended antenatal care in our setting, where syphilis screening is undertaken routinely and managed at the time of diagnosis. Additionally, the prevalence of maternal HIV infection (28%) in our study was similar to the population prevalence (29%)—which has remained stable over the past decade¹⁶—and none of the stillbirths were attributed to HIV infection, in agreement with the low risk of maternal HIV infection as an independent risk factor for stillbirth.⁴

A systematic review on the association between bacterial infection and stillbirths only identified 17 eligible studies,¹⁷ and estimated that between 4.1% (England, 1981–1996)

and 20·6% (Sweden, 1970–1975) of stillbirths were due to bacterial infections.^{18,19} Furthermore, only two case series from Africa and none from south Asia were identified,^{20,21} resulting in an inability to evaluate the role of bacterial infection as a cause of stillbirth in LMICs.¹⁷ In a study of 18 stillbirths in Mozambique²² that included culture of blood, cerebrospinal fluid, and lung samples as well as molecular diagnostic testing of lung tissue and complete diagnostic autopsy, four (22%) stillbirths were attributed to infection, with three due to group B streptococcus. Group B streptococcus was conservatively estimated to have caused at least 57 000 stillbirths globally in 2015, which was around two-thirds of deaths from invasive group B streptococcal disease in young infants (n=90 000).²³ The criteria used to attribute bacterial infections as a cause of stillbirth, however, differ between studies, and should be standardised. Nevertheless, our observations highlight the need for further research on the interaction of the maternal rectovaginal microbiome and the risk for adverse fetal outcomes such as invasive bacterial disease. This research could inform whether modulation of the vaginal microbiome could prevent bacterial-infection-related stillbirths, as suggested by the association between clindamycin treatment in women with abnormal vaginal flora at less than 22 weeks' gestation and the reduced risks of preterm birth and late miscarriage.²⁴

In McClure and colleagues' multicentre study,¹⁵ in which a computer-based hierarchical algorithm was used to assign cause of death from clinical registry data, 47% of stillbirths were attributed to intrauterine asphyxia, which occurred secondary to underlying maternal conditions such as prolonged labour (38%), antepartum haemorrhage (19%), and pre-eclampsia or eclampsia (18%). In our study, only 1% of stillbirths were attributed to intrauterine asphyxia (with unexplained pathogenesis). Nevertheless, in our study, similar to the observations by McClure and colleagues,¹⁵ there was a high prevalence of stillbirths attributed to hypertensive disorders (19%), antepartum haemorrhage (17%), and placental circulatory disorders (9%), which probably led to intrauterine asphyxia as the final event. Prolonged labour, although not a specific category in the SCRN classification, was present in 23% of women in our study—lower than the frequency of 38% reported by McClure and colleagues, which probably reflects differences in access to obstetric care between settings.¹⁵

The heterogeneity in cause of stillbirth attribution between our study and that by McClure and colleagues¹⁵ exemplifies the need for greater harmonisation in the classification of causes of stillbirths.⁶ In 2016, Flenady and colleagues²⁵ identified 81 different classification systems used to report on causes of stillbirths between 2009 and 2014, the majority developed in high-income countries and none considered to be ideal. Although the WHO International Classification of Diseases–Perinatal Mortality provides an opportunity to standardise reporting

across different settings, it is recognised to have limitations where more detailed investigation of stillbirths such as placental or fetal autopsy occur.²⁵

The inclusion of placental investigation in our study, generally absent from previous published studies from LMICs, resulted in 19% of stillbirths being attributed to pathological placental conditions, including circulatory disorders (ie, placental infarcts) and placental inflammation. The SCRN study⁷ reported that placental macroscopic and histological examination was the most informative test for investigating stillbirths, being useful for diagnosis in 64·6% of cases, thereby highlighting the importance of its inclusion in future studies investigating the causes of stillbirths.

Limitations of our study included the absence of methods shown in the SCRN study⁷ to be useful in identifying causes of stillbirths: complete autopsy diagnosis (useful in 42·4% of cases), karyotyping (11·9%), antiphospholipid antibody testing (1·1%), and fetal–maternal haemorrhage testing (6·4%). We also did not test for viruses such as parvovirus B, rubella, Coxsackie A or B virus, or cytomegalovirus, which have been implicated as causes of stillbirths.¹² Furthermore, we retrospectively abstracted maternal clinical information and information on the history of the labour from available medical records. The absence of the additional tests, coupled with possible incomplete medical records, could have contributed to no cause being identified for 18% of stillbirths in our study. The value-add of these extra tests should be considered for investigation in future studies of the causes of stillbirths in LMIC settings.

Another limitation of this study was that it was based in a hospital; however, this is unlikely to be a major factor in our setting, where most births (99%) occur in health facilities (appendix). Furthermore, we only enrolled 60% of all eligible stillbirths delivered at Chris Hani-Baragwanath Academic Hospital because of limiting enrolment to one or two cases per day to reach the a priori planned sample size of 300 cases; thus, the study findings are unlikely to have been systematically biased.

Although the results from this study provide important insight into the causes of stillbirth in settings such as ours, the results are not necessarily generalisable to less-resourced settings. For example, there might be a higher contribution of intrapartum obstetric causes of stillbirths in regions where interventions such as caesarean section, which would allow stillbirths consequent to prolonged labour or antepartum haemorrhage to be avoided, are less accessible. More studies that systematically investigate the causes of stillbirths in diverse LMIC settings are needed to provide insight into strategies, interventions, and research priorities that are warranted to achieve the Every Newborn Action Plan goal of reducing stillbirth rates to 12 per 1000 births by 2030.²

Contributors

SAM and CLC conceived the study, developed the protocol, and sourced funding. CLC, CB, and JW collected the data. SMa, SMo, RC, YA, and

SAM were responsible for ascertaining causes of stillbirth. AI, CLC, and SAM analysed the data. All authors were responsible for data interpretation, and provided input and reviewed the final manuscript. SAM did the first draft of the manuscript.

Declaration of interests

This was an investigator-initiated study that received financial support from Novartis Vaccines Division and GlaxoSmithKline (GSK) Biologicals SA (on March 2, 2015, Novartis' non-influenza vaccines business was acquired by the GSK group of companies). The authors received no financial support or other form of compensation related to the development of this manuscript.

Data sharing

The metadata from this study, which includes the individual de-identified participant data that underlie the results reported in this Article, will be available from the publication date on the Respiratory and Meningeal Pathogens Research Unit website (<http://www.rmpru.com>), along with the study protocol. The data will be password protected and made available to researchers wishing to further analyse the data.

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