UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

School Of Clinical Medicine

Infectious causes of stillbirths among women at Rahima Moosa Mother and Child Hospital

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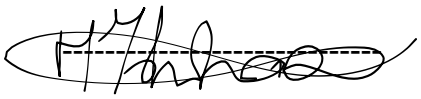
A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Obstetrics and Gynaecology

Johannesburg, 2024

DECLARATION

I, Dr Thendo Patricia Tshivhase, declare that this research report is my own, unaided work. It is being submitted for the degree of Master of Medicine (MMed) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

Date: December 2024

DEDICATION

This MMedis dedicated to my loving husband, Victor Fulufhelo Nwedamutswu and our beautiful son, Oritonda Wazwothe Nwedamutswu**.**

PRESENTATIONS

1. Accepted for International Federation of Gynaecology and Obstetrics (FIGO) Paris International conference, 9-13 October 2023, Poster presentation.

ABSTRACT

Background: Stillbirths are associated with socio-economic consequences including physical and mental impairment to the bereaved family as well as financial implications to the family and the health-care system. There is a close association of stillbirths in developing countries and infections, however, often without specific microorganisms identified.

Objectives: To determine the prevalence and causes of intrauterine infections by describing the microbiology, culture and sensitivity (MCS) as well as histopathological findings identified in placental samples of stillbirths

Methods: A retrospective, descriptive study was conducted at RMMCH, Johannesburg, (from 1 January 2020 to 31 December 2020) to review infections in placentas of stillborn fetuses of at least 22 weeks' gestational age or with a birth weight of ⩾500g. Medical records were used to abstract maternal clinical information. Microbiological and histopathological data of placentas were retrieved and assessed to identify their associations with chorioamnionitis as well as for assessment of microorganisms' drug sensitivity and resistance patterns. Statistical software (STATA) version 17 was utilized for data analysis. Descriptive data was reported using means, medians, and standard deviations.

Results: A total of 103 patient records were reviewed. Placental reports of 79/103 (76.7%) stillbirths were examined, with (33/79) 41.7% showing the presence of chorioamnionitis. The findings showed that 17 out of 33 cases (51.5%) had acute chorioamnionitis with a fetal inflammatory response. A total of 50/79 (63.3%) placental membranes were sent for analysis with 35 microorganisms cultured from 25 specimens. The incidence of the various organisms included: 9/35 (25.7%), *Staphylococcus (S) epidermidis*, 7/35 (20.0%) *Escherichia (E) coli*, 7/35 (14.3%) *Enterococcus faecalis*, 4/35 (11.4%) *Staphylococcus haemolyticus*, 3/35 (8.6%) *Staphylococcus aureus* and 3/35 (8.6%) *Group B Streptococcus* cases. Most of the Gram-negative organisms, namely *E coli* and *Klebsiella oxytoca* were 100% sensitive to amoxicillin -clavulanic acid, second, third and fourth generation cephalosporins (ceftriaxone, cefoxitin, cefuroxime and cefepime) and carbapenems (ertapenem, meropenem and imipenem). One case (2.9%) had Candida albicans cultured which was sensitive to fluconazole. No viruses or parasites were found in this study.

Conclusion: Chorioamnionitis was associated with 41.7% of stillbirths. *S. Epidermidis, E. coli and Enterococcus faecalis* were among the commonest microorganisms identified in this study with most of them being sensitive to amoxicillin-clavulanic acid, cephalosporins, ciprofloxacin and ertapenem. Routine investigation including placental histopathology and MCS of all stillbirths and poor fetal outcomes should be emphasized with prompt review of results and appropriate clinical follow-up.

Keywords: STILLBIRTH, INFECTION, CHORIOAMNIONITIS

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- To the National Health Laboratory Services and Corporate Data Warehouse team for all their efforts with access to histopathology and microbiology results.

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LIST OF ABBREVIATIONS

CDW- Corporate Data Warehouse

COVID-19 PCR- Coronavirus disease of 2019 Polymerase Chain Reaction

CMJAH- Charlotte Maxeke Johannesburg Academic Hospital

E-Escherichia

GBS- Group B Streptococcus

g/dL- Grams per decilitre

HELLP- Haemolysis, elevated liver enzymes and low platelet count

HIV-Human immunodeficiency virus

HJH- Helen Joseph Hospital

LNMP- Last Normal Menstrual Period

MCS-Microbiology, Culture and Sensitivity

NHLS- National Health Laboratory Services

Redcap-Research Electronic Data Capture

RMMCH-Rahima Moosa Mother and Child Hospital

S - Staphylococcus

SPP- Species plural

STATA- Statistical software

WHO- World Health Organization

WITS- University of the Witwatersrand

JOURNAL ARTICLE (SAJOG)

Title:

INFECTIOUS CAUSES OF STILLBIRTHS AMONG WOMEN AT RAHIMA MOOSA MOTHER AND CHILD HOSPITAL

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Conflict of interest:

The researcher and co-authors have no conflict of interest to declare.

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study with most of them being sensitive to amoxicillin-clavulanic acid, cephalosporins, ciprofloxacin and ertapenem. Routine investigation including placental histopathology and MCS of all stillbirths and poor fetal outcomes should be emphasized with prompt review of results and appropriate clinical follow-up.

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INTRODUCTION

Stillbirths are associated with socio-economic consequences including physical and mental impairment to the bereaved family as well as financial implications to the family and the health-care system.[1] An increased burden of stillbirths remains in low-to-middle income countries including Sub-Saharan Africa and South Asia.[1] South Africa is an upper-middle-income country and is greatly affected by this burden. The World Health Assembly approved the Every Newborn Action Plan (ENAP) with the aim of reducing stillbirths from 18.4 to 12 per 1000 births by 2030..[2]

Stillbirth is the end-result of contributory causes including maternal, fetal and placental factors which result in fetal demise.[3] According to the National Department of Health of South Africa, the stillbirth rate for Gauteng province in 2019 was 18.7/1000 total births.[4] Infections have been shown to be one of the main contributors to fetal death.[5, 6] About 16% of the stillbirths in low to middle income countries have been associated with infections although this has been mainly based on hospital audits and verbal autopsies without specific microorganisms identified.[1]

The definition of chorioamnionitis is fetal membrane inflammation, which includes both the chorion and the amnion.[7] Most cases of chorioamnionitis are subclinical, with insufficient clinical evidence at birth and needs evaluation of the placenta to be confirmed.[8] Histopathology and microbiological analysis of the placenta can be used to diagnose chorioamnionitis. Histopathology has been shown to be the gold standard.[6] The Global Alignment of Immunization safety assessment in pregnancy (GAIA) case definition for chorioamnionitis includes clinical diagnosis such as maternal fever, uterine tenderness, and fetal tachycardia along with confirmation by histopathology or positive culture at 22-0/7 weeks or greater gestational age.[9] More than 130 bacteria are associated with intra-uterine infections, many of these being associated with stillbirths.[10] The most frequently isolated bacterial microorganisms in intrauterine infections are *Ureaplasma species* (*Ureaplasma parvum* and *Ureaplasma urealyticum*), *Fusobacterium special plural (spp.), Streptococcus spp.*, and, less frequently, *Gardnerella spp., Mycoplasma spp.,* and *Bacteroides spp*.[5] Other studies have found *Escherichia (E) coli, Ureaplasma urealyticum* and Group B Streptococcus (GBS) to be more frequent.[11, 12]

Intrauterine microbial infection especially in placental tissue has been associated with adverse outcomes including preterm birth, fetal distress, stillbirth and adverse neurological

outcomes later in life.[13] Knowledge of the burden of disease and the responsible pathogens can assist in directing management strategies.

We aimed to describe the prevalence of chorioamnionitis in stillbirths delivered and to identify the common infectious causes and their characteristics.

METHODS

This was a retrospective, descriptive study describing the clinical presentation, placental microbiological and histopathological features of women who delivered stillborn babies from 1 January 2020 until 31 December 2020. The microbiology and histopathology results were obtained from the National Health Laboratory Services (NHLS) and analysed with corresponding maternal case files.

Setting: The study was conducted at Rahima Moosa Mother and Child Hospital (RMMCH), a teaching hospital located in Coronationville, Johannesburg, Gauteng province, South Africa. In 2019 there were 13 974 babies delivered of which 8881 (63.6%) were delivered vaginally and 5093 (36.4%) were caesarean section deliveries.[14] There were 210 stillbirths, 21.9% (46/210) were fresh stillbirths and 78.1% (164/210) were macerated stillbirths with a stillbirth rate of 15 per 1000 total births.[14] In 2020, there were 14 221 babies delivered with 227 stillbirths and a stillbirth rate of 16 per 1000 total births.[14] RMMCH is affiliated with a level 3 hospital, Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). All tissue samples, including placental samples that are collected are sent and processed at the National Health Laboratory Services (NHLS) laboratories at CMJAH.

Inclusion criteria: All women who delivered stillbirths in 2020 were included. The study was limited to women who delivered at least one stillborn fetus from 22 weeks' gestational age or a birthweight of ≥500 grams.

Exclusion criteria: There were no exclusion criteria in this study.

Sampling and sample size: A list of women who delivered stillbirths was obtained from birth records in the labour ward, antenatal wards, Obstetrics and Gynaecology wards and operating theatres. Of the 227 women who delivered a stillborn baby, the files of 103 (45.4%) who delivered 105 babies (including 2 sets of twins with one alive in each pregnancy) were

available for review. A total of 103 women and 103 stillborn were reviewed. A total of 76.7% (79/103) women had histopathology reports available for review of which 63.3% (50/79) had the microbiology available to review as well.

Measurements: Maternal and fetal clinical data were obtained from maternity records. Extracted data included patient demographics, age, parity, booking status, gestational age at delivery, mode of delivery and maternal outcomes. Placentas of women whose babies experience a poor fetal or neonatal outcome, including stillbirths, are routinely sent to the NHLS for microbiological and histological analysis. Results of the histopathological examination and MCS assessment were obtained from NHLS Corporate Data Warehouse (CDW).

Data analysis: REDCap (Research Electronic Data Capture) electronic data capture tools was used to capture and manage data. Provision of REDCap is made available by the University of the Witwatersrand (WITS). REDCap is a safe and reliable, web-based application made to facilitate the capturing of data for the purpose of research. Analysis of data was done with assistance of a biostatisticians at WITS. Statistical software (STATA) version 17 was used for data analysis. Descriptive data were expressed using means and medians with standard deviations.

Ethical considerations: The study was approved by the the University of the Witwatersrand's Human Research Ethics Committee (Medical), (Clearance certificate number M211102). Permission to conduct the study was granted by RMMCH.

RESULTS

A total of 103 (45.4%) out of 227 patient records of women who delivered stillbirths from 01 January 2020 to 31 December 2020 were available for review. Among the 103 women, 105 babies were delivered with 2 women delivering twin pregnancies and subsequently delivering one stillbirth each.

The mean age of patients was 29.1 years at the time of delivery with 28/103 (27.2%) patients having a parity of zero before delivery and 76 (73.8%) having a parity of one or more. Eighty-four (81.6%) women were booked and attended at least one antenatal care visit before they delivered. Gestational age was assessed using antenatal ultrasound assessment, last

normal menstrual period (LNMP) or symphysis-fundal height (SFH) in descending order as follows: (i) ultrasound performed prior to 24 weeks' gestation for 25/103 (24.3%) women, (ii) Last normal menstrual period for 69/103 (67.0%) women (iii) first palpation at booking for 8/103 (7.8%) women, and (iv) symphysis-fundal height at delivery for 1/103 (1.0%) woman. The overall median gestational age at delivery was 35-0/7 weeks with an interquartile range (IQR) of 31-0/7 to 38-0/7 weeks.

The median haemoglobin was 11.2g/dL with an IQR of 10.2 g/dL to 12.6 g/dL. Ninety-nine (96.1%) women were syphilis negative and one (1.0%) of woman was syphilis positive and completed Penicillin G Benzathine treatment 15 weeks prior to the delivery. Three (2.9%) of the patients did not have a syphilis test done. Ninety-four (91.3%) women were rhesus positive, two (1.9%) were rhesus negative, one with no antibodies detected throughout pregnancy and one with unknown antibodies. Seven (6.8%) patients had unknown rhesus status. Twenty/103 (19.4%) women were Human immunodeficiency virus (HIV) positive and 19/20 (95.5%) were on antiretroviral treatment. Of the 19/20 that were on treatment, 17/20 (89.5%) were on Tenofovir/Lamivudine/ Dolutegravir fixed dose combination and 2/20 (10.5%) on Tenofovir/Emtricitabine/Efavirenz fixed dose combination. A CD4 count was done on 14 (70.0%) of the 20 HIV positive women. The latest (test done within 6 months of delivery) median CD4 count was 281 (221-480) cells per microlitre. Eleven (55.0%) out of the 20 HIV positive women had their viral load done during pregnancy with 6 /11 (30.0%) women having had a viral load that was lower than detectable recorded, that is, less than 20 copies per millilitre.

Three (2.9%) women were Coronavirus disease of 2019 Polymerase Chain Reaction (COVID PCR) positive at delivery, 32/103 (31.1%) were negative and 50/103 (48.5%) had undocumented COVID status at delivery. Eighteen (17.5%) had no COVID test done since they delivered before the pandemic testing for COVID PCR in South Africa (March 2020).

Twenty-six (25%) women had associated comorbidities including hypertension, gestational diabetes and asthma. Nineteen (18.5%) of the women had complicated hypertensive disorders of pregnancy including preeclampsia, eclampsia and haemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome. Five (4.9%) women were diagnosed clinically with an infection at the time of the stillbirth. Three of the five cases had clinical chorioamnionitis and two had a urinary tract infection. The occurrence of clinical chorioamnionitis was markedly lower compared to the findings of chorioamnionitis from histopathology and microbiology

results. Ninety-eight (95.1%) of the women were clinically well post-partum. Two (1.9%) patients had puerperal sepsis, one diagnosed with endometritis and the other with pyelonephritis, both recovered and were discharged. Three (2.9%) of the women had undocumented outcome statuses. No maternal deaths were recorded from the 103 women reviewed.

Table 1*: Characteristics of women who delivered stillborn infants*

Variable	**Category**	**N=103, n (%) [range]**
Age in years	Mean (SD)	29.1 (6.6) [range]
Race	Black	95 (92.2)
	Caucasian	1 (1.0)
	Coloured	6 (5.8)
	Indian	1 (1.0)
Gravidity	One	27 (26.2)
	Two	20 (19.4)
	Three	33 (32.0)
	Four	15 (14.6)
	Five or more	8 (7.8)
Parity	Zero	28 (27.2)
	One	23 (22.3)
	Two	34 (33.0)
	Three	14 (13.6)
	Four or more	4 (3.9)
Ascertainment of GA		
First Ultrasound (before 24 weeks)		25 (24.3)
LNMP		69 (67.0)
First palpation		8 (7.7)
SFH at delivery		1 (1.0))
Gestational age in weeks		
LMNP	Median (IQR)	36.3 (32.0 – 38.4)
First Ultrasound	Median (IQR)	33.3 (29.0 – 35.6)
First palpation	Median (IQR)	35.0 (29.1 – 38.0)
SFH	Median (IQR)	34.0 (34.0 – 34.6)
Overall gestational age	Median (IQR)	35.0 (31.0-38.0)
Antenatal care	Booked	84 (81.6)

	Un-booked	19 (18.4)
Haemoglobin (g/dl) at booking	Median (IQR)	12.1 (10.8 – 13.0)
Haemoglobin (g/dl) before/at delivery	Median (IQR)	11.2 (10.2 – 12.6)
Syphilis	Positive	1 (1.00)
	Negative	99 (96,1)
	Not done	3 (2.9)
Titre RPR (positive) (n=1)	1:8	1
Syphilis treatment (n=1)	Penicillin G Benzathine	1
Rhesus status	Positive	94 (91.3)
	Negative	2 (1.9)
	Unknown	7 (6.8)
Antibodies if Rhesus negative (n=2)	Absent	1
	Unknown	1
COVID-19 Rapid/PCR	Positive	3 (2.9)
	Negative	32 (31.1)
	Unknown	50 (48.5)
	Not done	18 (17.5)
HIV status	Positive	20 (19.4)
	Negative	81 (78.6)
	Unknown	2 (1.9)
CD4+ test (HIV positive, n=20)	Done	14 (70.0)
	Not done	6 (30.0)
CD4+ count (mm^3)	Median (IQR)	281 (221 – 480)
Last viral load (copies/mm^3) recorded (HIV positive, n=20)	Lower than detectable	6 (30.0)
	Unknown	9 (45.0)
	> 20	5 (25.0)
Viral load (if >20 copies/mm^3) N=5	Median (IQR)	16810 (901 – 20800)
HbsAg (HIV positive, n=20)	Positive	0 (0.0)

	Negative	17 (85)
	Unknown	3 (15)
On ART (HIV positive, n=20)	Yes	19 (95.0)
	No	1 (5.0)
ART (n=19)	Tenofovir/Emtricitabine/ Efavirenz (TEE)	2 (10.5)
	Tenofovir/ Lamivudine/Dolutegravir (TLD)	17 (89.5)
Comorbidities		
	Chronic hypertension	0
	PIH, PET/HELLP syndrome	19 (18.4)
	Diabetes mellitus type 1	0
	Diabetes mellitus type 2	3 (2.9)
	Gestational diabetes mellitus	2 (1.9)
	Autoimmune disease	0
	Asthma	2 (1.9)
	None	77 (74.8)
Presence of infection (clinical assessment)	Yes	5 (4.9)
	No	98 (95.1)
Infections (n=5)	Chorioamnionitis	3 (60.0)
	UTI	2 (40.0)
Other infections during pregnancy	Yes	1 (1.0)
	No	101 (98.0)
	Unknown	1 (1.0)
If yes, diagnosis	Syphilis	1
Diagnosis of stillborn	Antepartum (before onset of labour)	58 (56.3)
	Intrapartum (during labour)	34 (33.0)
	At delivery	11 (10.7)
Cause of intrapartum stillborn (n = 34)	Fetal distress	5 (14.7)
	Cord prolapse	1 (2.9)
	Placental abruption	10 (29.4)
	Uterine rupture	2 (5.9)
	Unknown	16 (47.1)

ART- Antiretroviral, SD- Standard deviation, IQR-Interquartile range, GA- gestational age, LNMP- last normal menstrual period, SFH- symphysis fundal height, g/dl- grams per decilitre, RPR- rapid plasma reagin, HIV- human immunodeficiency virus, CD4- clusters of differentiation 4, mm^3 – cubic millimetre, HbsAg- hepatitis B surface antigen, PIH- pregnancy-induced hypertension, PET- preeclampsia, HELLP- haemolysis, elevated liver enzymes and low platelet count, TEE- tenofovir/emtricitabine/efavirenz, TLD- tenofovir/lamivudine/dolutegravir

A total of 105 babies were delivered to 103 women with 2 (2.0%) sets of twins with one alive baby in each pregnancy. Of the 103 stillbirths included in this study, 28 (27.2%) were fresh stillbirths and 75 (72.8%) were macerated stillbirths. Among these, 58/103 (56.3%) were diagnosed antepartum, 34/103 (33.0%) intrapartum and 11/103 (10.7%) immediately following delivery. Intrapartum complications included five (14.7%) with fetal distress, one (2.9%) cord prolapse, 10 (29.4%) placental abruption, two (5.9%) with a uterine rupture and 16 (47.1%) unknown causes. Eighty-three (80.6%) patients were delivered vaginally and 20 (19.6%) delivered via caesarean section. Indications for caesarean section delivery included 6 (30.0%) for previous caesarean section (two in labour), 5 (25.0%) for fetal distress, 4 (20.0%) for prolonged labour and 1 (5.0%) patient who had a failed induction of labour. For four (20.0%) of the patients, we could not find the indication for caesarean section delivery due to incomplete/missing notes.

Among the stillborn babies, 62 (60.2%) were male, 40 (38.8%) were female and one (1.0%) was 560 grams (g) macerated stillborn baby with sex undocumented. The median birthweight was 2190g with an IQR of 1520- 2960g.

Table 2: Characteristics of stillborn infants who were delivered

Variable	**Category**	**N=103, n (%) [range]**
Mode of delivery	Vaginal delivery	83 (80.6)
	Caesarean section	20 (19.6)
If caesarean section (n=20)	Elective	0 (0.0)
	Emergency	20 (100)

Indication for caesarean section (n=20)	Fetal distress	5 (25.0)
	Prolonged labour	4 (20.0)
	Previous caesarean section	6 (30.0)
	Failed induction of labour	1 (5.0)
	Unknown	4 (20.0)
Number of fetuses	Singleton	101 (98.0)
	Twins	2 (2.0)
Sex	Male	62 (60.2)
	Female	40 (38.8)
	Unknown	1 (1.0)
Birthweight (grams)	Median (Interquartile range)	2190 (1520 – 2960)
Type of stillborn	Fresh stillborn	28 (27.2)
	Macerated stillborn	75 (72.8)

Seventy-nine out of the 103 women (76.7%) had their placentas submitted for histopathological assessment, and of those, 50/79 (63.3%) had placental membranes sent for MCS as well. Placental lesions were categorized according to the Amsterdam Placental Workshop Group's consensus definitions of placental lesions.[15] The mean placental weight was 384g (182g-589g) for the median gestational age of 35-0/7 weeks. Cord insertion was parenchymal in seventy- three (70.9%) cases. Of the 79 cases, cord abnormalities included 13 (16.5%) cases of funisitis, four (5.1%) cases of hypercoiling, three (3.8%) cases of cord thrombosis and one (1.0%) had a two-vessel cord.

Meconium staining was present in 12 (15.2%) placentas. Macroscopic infarction was seen in 32 (40.5%) placentas.

A total of thirty-three (41.8%) placentas showed evidence of chorioamnionitis with 32 (97.0%) of these categorized as acute chorioamnionitis. A maternal response was present in all placentas that showed evidence of chorioamnionitis. Nineteen (57.6%) specimens that showed chorioamnionitis were from preterm deliveries (< 37 completed weeks) and 14/33 (42.4%) were from term deliveries. A fetal response was present in 17 cases (51.5%) of chorioamnionitis and consisted mainly of stage 1 and grade 1 fetal response. Thirty-two (40.5%) specimens showed evidence of maternal vascular malperfusion with 8 (10.1%) cases showing both placental insufficiency and chorioamnionitis.

Figure 1: Photomicrographs of sections of placental membranes of women who delivered Courtesy of Prof. R Wadee.

Haematoxylin and eosin-stained section of placental membranes demonstrating: (a) chorioamnionitis (original magnification × 100); (b) Funisitis (original magnification × 100).

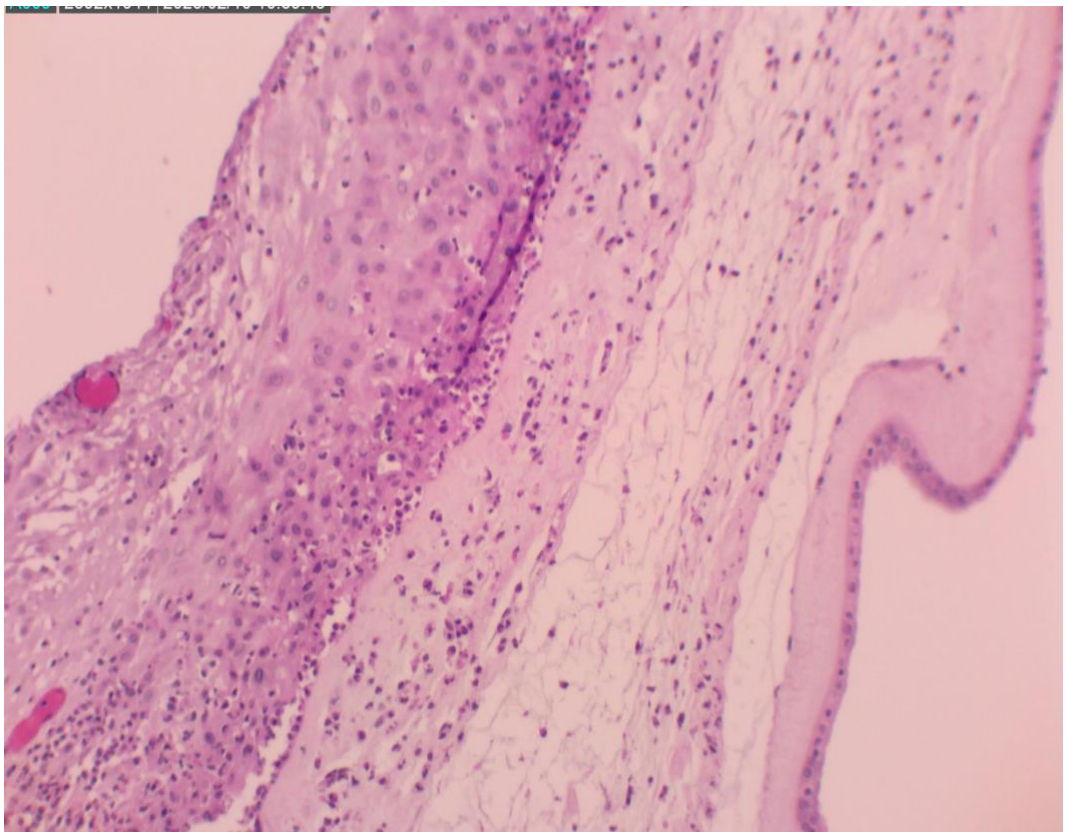


Figure 1(a)

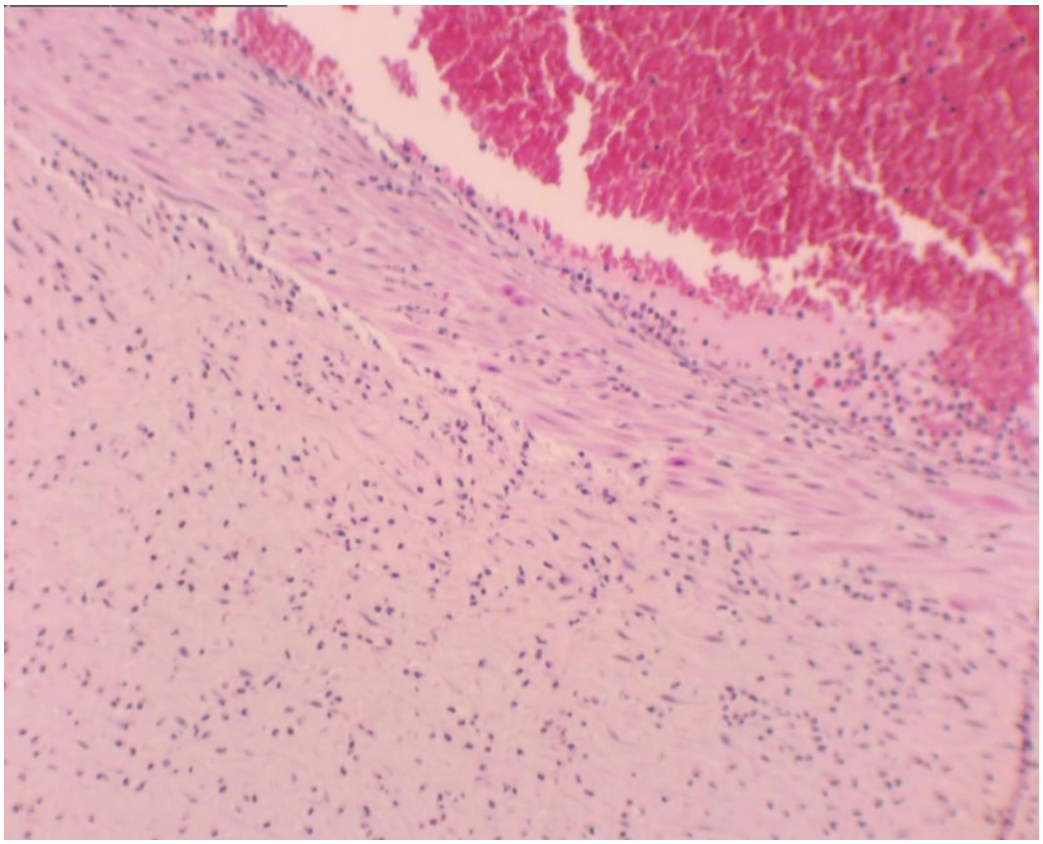


Figure 1(b)

Out of the 79 placentas submitted for histopathological analysis; 50 (63.3%) cases also had placental membranes sent for MCS. Bacteria was observed in 25/50 (50.0%) cases of the placental membranes. Some specimens cultured more than one microorganism with a total of 35 microorganisms identified in this study. Twenty-two (44.0%) specimens had no bacteria observed, two (4.0%) specimens were contaminated, and one (2%) specimen was rejected due to incomplete seal and leakage. Of the 25 placental membrane specimens that cultured microorganisms, 13/25 (52.0%) had evidence of chorioamnionitis on histopathology and 12 (48.0%) had bacteria cultured without evidence of chorioamnionitis.

Nine pathogenic microorganisms were observed with a total of 35 microorganisms from 25 specimens cultured. There were 9/35 (25.7%) cases of *Staphylococcus epidermidis*, 7/35 (20.0%) *Escherichia (E) coli*, 5/35 (14.3%) *Enterococcus faecalis*, 4/35 (11.4%) *Staphylococcus haemolyticus*, 3/35 (8.6%) *Staphylococcus aureus*, 3/35 (8.6%) *Streptococcus Agalactiae*, 1/35 (2.9%) *Citrobacter koseri*, 1/35 (2.9%) *Staphylococcus Lugdunensis*, 1/35

(1.0%) *Klebsiella Oxytoca* and 1/35 (2.9%) Candida albicans. Most Gram-positive organisms including *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylo*coccus *haemolyticus* showed 100% sensitivity to ciprofloxacin, rifampicin and vancomycin. The majority of Gram-negative organisms, namely *E. coli* and *Klebsiella oxytoca* were 100% sensitive amoxicillin -clavulanic acid, second, third and fourth generation cephalosporins (ceftriaxone, cefoxitin, cefuroxime and cefepime) and carbapenems (ertapenem, meropenem and imipenem). Both Gram- positive and Gram- negative organisms were resistant to ampicillin (44.4%) and trimethoprim- sulfamethoxazole (30.8%). One (2.9%) *Candida albicans* was cultured and sensitive to fluconazole and amphotericin B.

Figure 2: Flowchart showing numbers of placenta specimens sent for analysis for stillborn infants

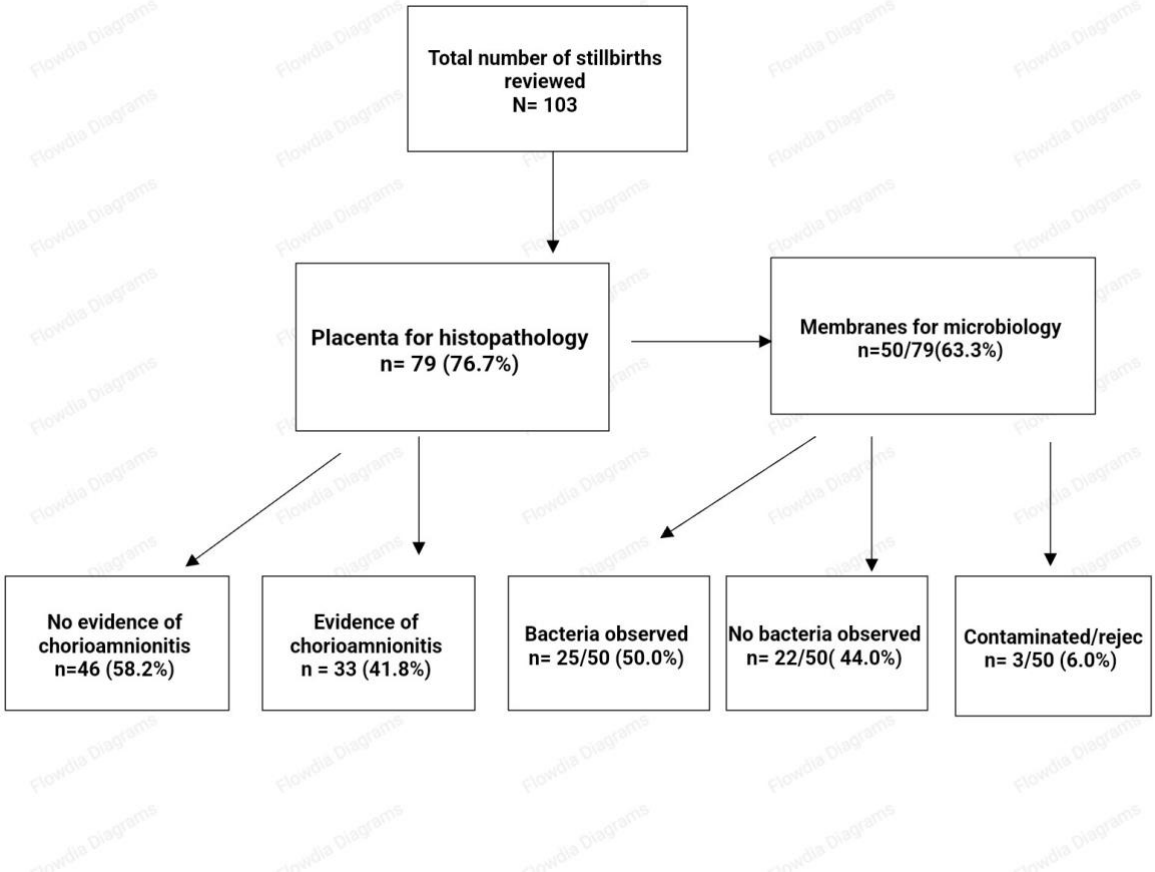
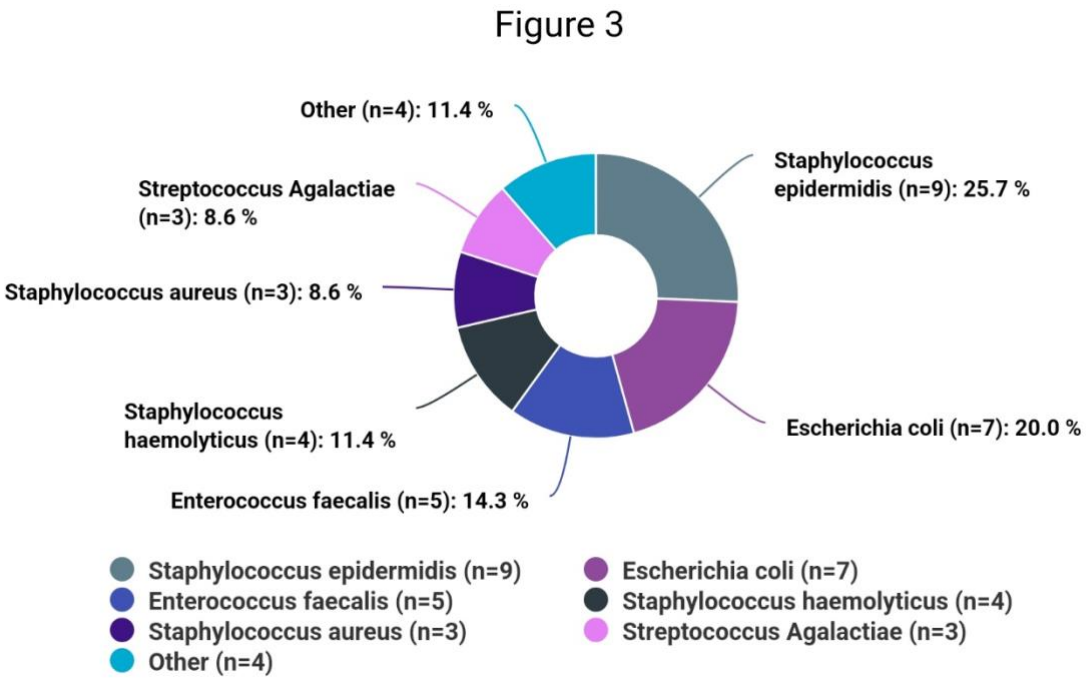


Figure 3: Microbiological culture results of placental membranes collected from women who delivered a stillborn infant



DISCUSSION

Infections, whether maternal or fetal, remain one of the commonest causes for stillbirths in low- and middle-income countries (LMIC) as well as South Africa, which is an upper middle-income country.[6] Chorioamnionitis as a cause of stillbirths is often underdiagnosed in LMICs as it may be sub-clinical, and placental histology is not conducted due to limited resources.[16]

Our study showed that 41.8% of stillbirths are associated with chorioamnionitis which was similar to findings from a Cape Town and a Zimbabwean study [17] which diagnosed chorioamnionitis on histopathology in 34% of women presenting with a stillbirth. These results show a higher prevalence compared to Soweto, which is a similar setting, which showed an association in 25%. [1,12] Many factors may contribute to the disparities of the prevalence such as resources, study population, criteria for placenta evaluation, and antibiotic protocols of health care facilities. The socio- economic status, level of expertise, availability of trained health care practitioners may also influence the prevalence of chorioamnionitis.[12] This study has shown poor correlation between clinically suspected chorioamnionitis and histologically confirmed chorioamnionitis as only 3/103 (2.9%) cases were identified clinically while there were 33/79 cases (41.8%) diagnosed histologically; this poor concordance is in line with the literature.[15,18] This may be because some patients present with subclinical chorioamnionitis. Chorioamnionitis was confirmed histopathologically in all 3 cases of clinically diagnosed chorioamnionitis cases. Of those who had chorioamnionitis on histopathology 19/33 (57.6%) were preterm (before 37 weeks) deliveries and 14/33 (42.4%) were term deliveries. Sixty-six (64.1%) of stillbirths in this study were delivered preterm. Diagnosis of clinical chorioamnionitis requires an increased level of suspicion on the part of the health care professionals. A local study revealed that health care professionals including midwives lacked adequate knowledge regarding screening, diagnosis and management of chorioamnionitis.[19] Routine surveillance and antibiotics have been shown to be effective in decreasing chorioamnionitis incidence in women with preterm rupture of membranes but not in those in active preterm labour with intact membranes.[20] A total of 17/33 cases of chorioamnionitis (51.5%) showed a fetal response including funisitis, vasculitis as well as and intervillositis which suggests that the infection occurred prior to fetal demise. Chorioamnionitis may be present in almost all cases of funisitis while funisitis is present in up to 60% cases of chorioamnionitis.[20] Our results are similar to findings by Malusi et al, which showed a fetal response of 49.5%.[17] Villitis with an unspecified cause was

documented in two (2.5%) of the 79 placentas examined and this has an increased risk of stillbirth recurrence in future pregnancies if the aetiology of the villitis is unknown.[21]

The HIV prevalence in this study was 19.4% - in keeping with the hospital's statistics of 21.7% [14], while the national prevalence of HIV in South Africa is 30.0%. [22] Only four of the 20 HIV positive patients showed evidence of chorioamnionitis on histopathology. A study by Ocheke et al showed comparable results regarding association of chorioamnionitis in HIV positive and HIV negative patients. [23] Another study by Ladner et al showed no statistical association between chorioamnionitis and HIV status. [24]

Bacterial infection and the subsequent inflammatory response have been shown to be responsible for most cases of chorioamnionitis.[25] The most common bacteria identified included *S. Epidermidis, E. coli* and *Enterococcus faecalis.* These findings were similar to two studies. [11,12] Madhi et al showed bacteria responsible for invasive fetal infection were group B *streptococcus, E coli, Enterococcus faecalis* and *S aureus***.**[1] This similarity helps improve understanding of the microbiology related to stillbirths and enables us to address the issue more effectively. Some studies have identified GBS, *Listeria monocytogenes and P.aeruginosa* as common isolates in placentas. [1, 26] A study done at RMMCH by Orji et al reviewing urinary tract infections during pregnancy and associated microorganisms found that *E. coli* was the commonest bacteria cultured followed by *Klebsiella species, Enterococcus faecalis* and *coagulase negative Staphylococcus* and these microorganisms showed comparable antimicrobial sensitivity to our study, primarily cephalexin, cefuroxime and ceftriaxone.[27] It is important to note that finding an organism does not prove causality *per se* although some of these bacteria have been cultured in neonates with severe sepsis.[28] Most organisms associated with these infections are often colonized in the gastrointestinal tract of pregnant women which acts as a reservoir for such organisms.[29,30] Our research indicates the importance of further investigations needed on the relationship between maternal rectovaginal microbiome and the potential adverse outcome on fetuses including invasive bacterial disease.

Despite chorioamnionitis being common there have been limited data on effective antimicrobial regimens to treat chorioamnionitis and prevent adverse outcomes. The majority of the identified microorganisms were sensitive to amoxicillin-clavulanic acid, cefepime, ceftriaxone, cefuroxime, ciprofloxacin and clindamycin. Most bacteria were resistant to

ampicillin, cloxacillin and trimethoprim- sulfamethoxazole (cotrimoxazole). Several randomized control trial studies have evaluated the efficacy of antibiotics in women with clinical chorioamnionitis and interventions using ceftriaxone, clarithromycin, and metronidazole were shown to be effective against most microorganisms.[20,31] A few studies recommend use of aminopenicillin, gentamicin, clindamycin or metronidazole to treat chorioamnionitis and erythromycin, vancomycin or clindamycin in penicillin-allergic patients.[20,31] The American College of Obstetrics and Gynaecology [32] recommends use of ampicillin and gentamicin to treat intraamniotic infections. The current WITS guidelines recommend ampicillin 1 gram 6 hourly with metronidazole 400 milligrams 8 hourly to be continued for 5 days post-delivery.[33] There have been concerns with regards to lack of data pertaining to antimicrobial use in South African public hospitals compounded by increasing antimicrobial resistance.[34] Similar to Orji et al, ampicillin, which is one of the commonest drugs used to treat infections in pregnancy, had the highest resistant pattern (45.8%) in this study.[27]

There is difficulty ascertaining if the placental specimens submitted for microbiological examination were collected in a sterile manner to minimize contamination as some of the bacteria identified are in fact normal bacterial commensals in skin, the gastrointestinal and vaginal tracts. Additionally, a greater number of specimens were sent for histopathological analysis in comparison to microbiological analysis. If more microbiological specimens had been submitted, it might have led to the identification of other species or sensitivities to antimicrobials that were not revealed in this study. It is important to note that causes of stillbirths may be multifactorial as some of the histology results showed both evidence of maternal vascular malperfusion as well as chorioamnionitis. Common viruses and parasites found in endemic parts of the country causing intra-uterine infections and associated with stillbirths including parvovirus B19 and malaria [6] were not found in this study.

LIMITATIONS

This study was limited to one hospital. Less than half of the stillbirth case records were available for review in the study period and therefore may not be representative of the population.

RECOMMENDATIONS

- Routine investigations, including placental histology and MCS of all stillbirths delivered or poor neonatal outcomes should be undertaken and there should be appropriate clinical follow up of mothers of babies with poor fetal outcome to allow for prompt review of the results as well appropriate management of infections, and for interventions in the planning of future pregnancies.
- There needs to be a high index of suspicion for chorioamnionitis in patients presenting to our facility as most patients go undetected and this results in adverse fetal outcomes.
- Antimicrobial stewardship needs to be reviewed and possibly revised in our setting as there is increased resistance patterns with common antibiotics used.
- A need for prospective studies with a large population to better understand the effects of intra-uterine infections and pregnancy our setting.

CONCLUSION

This study provides insight into the prevalence of infections associated with stillbirths in our setting. Chorioamnionitis was associated with 41.8% stillbirths and *S. Epidermidis*, *E. coli* and *E faecalis* were among the commonest microorganisms identified in this study with majority of them being sensitive to amoxicillin-clavulanic acid, cephalosporins, ciprofloxacin and clindamycin. Histopathological examination of placentas provides valuable information in identifying infection as a cause of stillbirth. The prevalence of stillbirth can be reduced in large part by additional research that compares the relationship between infections and stillbirths and further develops strategies to reduce adverse outcomes.

DISCLOSURE

The authors report no conflict of interest in this study.