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Focus and Scope

The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

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Editorial Commentary

Sweet Bitterness: Diabetes Mellitus and the Assault on Man's Dignity and Fecundity

Nwangwa, EK

Being an abstract of the Inaugural lecture delivered on Thursday, July18, 2024

In his seminal 107th inaugural lecture delivered at Delta State University, Abraka, Prof. Eze Kingsley Nwangwa embarks on a deeply personal and scientifically rigorous journey to interrogate the dual menace of diabetes mellitus and male reproductive dysfunction. Framed under the thought-provoking title *"Sweet Bitterness: Unmasking the Thief of Man's Dignity and Fecundity"*, the lecture offers a compelling exposition on the socio-medical implications of diabetes, especially its understudied impact on human fertility, fecundity, and quality of life.

The Bitter Aftertaste of Sweetness: Setting the Context

The lecture begins on a reflective note, rooted in the biblical charge to "be fruitful and multiply" (Genesis 1:28), and how the rise of lifestyle-related metabolic diseases such as diabetes mellitus has become a stark contradiction to this divine command. The inaugural Lecturer's early clinical encounters with diabetic patients; ranging from a cachectic child who was his patient at the early medical training to an elderly man's passionate resistance to castration in cancer care who preferred to die rather than losing his libido, serve as a springboard into his life-long research interest in diabetes and endocrine cum reproductive physiology.

The lecture's title, *Sweet Bitterness*, metaphorically contrasts the pleasurable allure of sugar and sweetness with the painful consequences of diabetes; a disease that literally sweetens the body's fluids while bittering its victims' lives through multi-organ complications and reproductive impairment.

Unmasking the Thief: Understanding Diabetes and Its Spread

The inaugural lecturer traces the history of diabetes mellitus treatment from ancient times to the landmark discovery of insulin in 1921. He defines diabetes as a chronic metabolic disease marked by hyperglycemia due to either insulin deficiency (Type 1) or insulin resistance (Type 2). Alarmingly, Nigeria is now home to over 11.2 million diabetics; the highest in sub-Saharan Africa, with urbanization, unhealthy diets, obesity, and sedentary lifestyles fuelling this epidemic.

The financial cost of diabetes in Nigeria has risen by almost 400% in a decade, burdening individuals and healthcare systems alike. Beyond economics, he emphasizes the social and emotional toll, particularly in the context of reproduction, where diabetes erodes not just physiological function, but the very essence of manhood and parenthood.

From Pancreas to Penis: Bridging Endocrinology and Reproductive Physiology

A large portion of the lecture is dedicated to elucidating the connection between diabetes and reproductive dysfunction, especially in men. Drawing on both foundational physiology and his own experimental work, Prof. Nwangwa explains

the role of the Hypothalamic-Pituitary-Gonadal (HPG) axis in maintaining reproductive health and how diabetes disrupts this axis, leading to hypogonadism, erectile dysfunction, sperm abnormalities, and ultimately infertility.

In experimental diabetic rats, his studies revealed alarming reductions in testosterone, LH, and FSH levels, alongside marked degeneration in the testes, pituitary, and pancreas. Semen analysis from diabetic subjects consistently showed lower sperm count, poor motility, and abnormal morphology; findings that have profound implications for human fertility.

The Emotional and Social Undercurrents

The lecture also addresses the psychosocial dimensions of infertility, particularly the stigma, shame, and psychological stress that accompany reproductive failure. Stress itself, Prof. Nwangwa notes, further impairs fertility via the Hypothalamic-Pituitary-Adrenal axis. The cyclical interaction between physiological dysfunction and emotional distress underscores the need for holistic care.

Equally concerning are the effects of endocrine-disrupting chemicals (EDCs), like phthalates, found in everyday products, which mimic or block natural hormones, exacerbating fertility issues. The inaugural lecturer's research findings confirm the testicular toxicity of such chemicals, reinforcing the call for public health interventions and regulation.

Therapeutic Innovations and Lifestyle Interventions

Rather than paint a picture of doom, the lecturer offers hope through research-backed lifestyle and dietary interventions. His studies highlight the benefits of intermittent fasting and exercise in improving glycemic control and restoring reproductive hormone balance. Additionally, the antioxidant properties of coconut water and vitamin E were found to ameliorate testicular damage and improve fertility outcomes in diabetic rats.

He also provides evidence that while pharmacologic agents like sildenafil are helpful in managing erectile dysfunction, natural food supplements and plant-based therapies offer promising complementary options. These include ginseng, L-arginine, black ginger, and even dietary habits involving fruits like watermelon and pomegranate.

Conclusion: A Call to Reclaim Man's Dignity and Fecundity

Prof. Nwangwa concludes by reiterating his core thesis: that the lifestyle-induced sweetness of modern life is exacting a bitter cost on human health, dignity, and the divine imperative of fruitfulness. Diabetes, he asserts, is more than a metabolic disease; it is a silent saboteur of fecundity, family continuity, and societal stability.

This lecture is more than a scholarly exposition; it is a clarion call to clinicians, researchers, policymakers, and the general public to confront the diabetes epidemic not just as a medical issue, but as a moral and existential threat to human reproduction. Prof. Nwangwa's contribution, rooted in decades of research, advocacy, and personal conviction, offers a roadmap for reclaiming man's biological and societal roles in a rapidly changing world.

Nwangwa EK. Sweet Bitterness: Diabetes Mellitus and the Assault on Man's Dignity and Fecundity. Afr. J. Trop. Med. & Biomed. Res. 2025; 8(1) 7-8
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Original articles

The Impact of Traditional Practices and Customs on Pregnant Women and Newborn Mothers' Healthcare-Seeking Behaviors at Primary, Secondary, and Tertiary Health Levels

Rennie V[1], Rishad A[2] & Emmanuel F[3]

ABSTRACT

Introduction: This study investigates the influence of traditional practices and customs on healthcare-seeking behaviors among pregnant women and newborn mothers across different healthcare levels in Bo District, Sierra Leone. Understanding the impact of these customs is crucial for developing culturally sensitive maternal and neonatal health strategies.

Materials and Methods: A mixed-methods approach was applied, involving 500 participants (pregnant women and newborn mothers) from urban and rural settings. Data was collected using the Ona mobile application through structured surveys. Quantitative analysis included descriptive statistics, Chi-Square tests, ANOVA, and multinomial logistic regression to evaluate primary, secondary, and tertiary healthcare utilization. The qualitative analysis explored the cultural context of healthcare decisions.

Results: Traditional practices were acknowledged by 52.40% of respondents, and 40.40% actively participated in customs such as herbal remedies, ceremonies, and traditional birthing practices. These practices significantly influenced the preference for primary (OR = 2.45, $p < 0.01$) and secondary (OR = 1.78, $p < 0.05$) healthcare facilities. Regression analysis indicated that integrating traditional practices into healthcare policies positively impacted healthcare-seeking behavior ($\beta = 0.42$, $p < 0.01$).

Conclusions: Traditional beliefs play a significant role in healthcare-seeking behavior, particularly at primary and secondary health levels. Integrating these practices into modern healthcare policies is essential for improving maternal and newborn health outcomes and fostering a more culturally sensitive healthcare system.

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INTRODUCTION

Healthcare-seeking behaviour among pregnant women and newborn mothers is shaped by a variety of factors, including cultural beliefs, traditional practices, and access to modern healthcare. In Sub-Saharan Africa, where traditional customs are deeply ingrained, these practices often play a significant role in determining when and where women seek healthcare [1]. Sierra Leone, particularly the Bo District, presents a case where these customs continue to influence maternal healthcare choices, despite ongoing efforts to promote modern medical practices.

Traditional practices and customs have long

dictated maternal healthcare behaviours in Sierra Leone. Many pregnant women and newborn mothers rely on traditional birth attendants (TBAs) due to their accessibility, affordability, and alignment with cultural values [2]. These traditional healers provide care based on long-standing customs trusted within the community, making them a preferred option for many women, especially in rural areas.

Herbal remedies, dietary restrictions, and traditional ceremonies related to pregnancy and childbirth are commonly practiced across Sierra Leone, including in Bo District [3]. These customs, while culturally important, often lead to delays in seeking formal healthcare services. Such delays can result in adverse maternal and neonatal outcomes, particularly when complications arise that require medical interventions beyond the capabilities of TBAs.

Cultural norms, such as the need for women to seek permission from husbands or elder family members before accessing healthcare, also contribute to healthcare delays [4]. Additionally, the fear of stigmatization related to pregnancy and childbirth, combined with a mistrust of modern medicine, can further limit healthcare utilization. These factors underscore the complexity of integrating traditional and modern healthcare practices to improve maternal and newborn health.

This study therefore

1. Identified and analyzed the traditional practices and customs influencing healthcare -seeking behaviours among pregnant women and newborn mothers in Sierra Leone.
2. Evaluated the differences in healthcare-seeking behaviours at primary, secondary, and tertiary healthcare levels due to traditional practices.

METHODS

Study Design

The research applied the mixed-methods approach, combining quantitative and qualitative data to gather comprehensive data on the impact of traditional practices and customs on healthcare-seeking behaviours among pregnant women and newborn mothers.

Participants' Selection and Exclusion Criteria

The criteria included:
Participants must reside in Bo District, Sierra Leone, be either pregnant women or newborn mothers, and must attend either the Bo Government Hospital (urban) or the Tikonko Village Health Centre (rural).

The study excluded women who had not lived in Bo District for at least six months or who were neither pregnant nor mothers of children under five. Also excluded were those who had not used services at the Bo Government Hospital or Tikonko Village Health Centre, and those unable to give informed consent due to communication or cognitive challenges. Participation was entirely voluntary, so anyone who chose not to take part or later withdrew was respectfully excluded.

Sample Size Determination

The following Cochran's formula was used in determining the sample size:

$$n_0 = \frac{Z^2 \cdot p \cdot (1 - p)}{e^2}$$

Where:

- $\mathbf{n_0}$ = initial sample size (before adjustments)
- **Z** = Z-value (standard normal deviate corresponding to the desired confidence level of 1.96 at 95% confidence)
- **p** = estimated proportion of the attribute

present in the population (set to 0.5 for maximum variability)
e = margin of error (0.05)

The study initially determined a sample size of 370 participants using Cochran's formula (state the formula and variables). To enhance the research's robustness, this number was increased by 26%, adding 130 participants, resulting in a final sample size of 500 - 294 pregnant women and 206 mothers of newborns. The adjustment accounted for potential non-responses, improved population representativeness, and reduced sampling errors, ensured adequate statistical power, and captured a broader spectrum of population variability, thereby enhancing the wider application of the study findings. The study employed a simple random sampling approach.

Data Collection
The study collected data from pregnant women and new mothers at primary, secondary, and tertiary health facilities in Bo District. Questionnaires were administered to the study participants via Ona, a mobile application. The study obtained ethical approval, ensured participants' confidentiality and anonymity, and encouraged honest responses. Ten data enumerators were trained before deployment in the field to collect data.

Piloting and Refining the Questionnaire
A pilot study was done with a small subset of participants to test the questionnaire's clarity, relevance, and reliability. Necessary adjustments were made. Based on the participants' feedback, the study ensured that the final questionnaire effectively captured the required data.

Monitoring and Adjusting
The participant selection process was monitored to ensure that the sample remained representative of the target population throughout the data collection process. Recruitment strategies were adjusted to ascertain the inclusion of socio-demographic groups that were underrepresented.

Statistical Analysis
A combination of qualitative and quantitative advanced statistical methods was used to analyze the research data. Advanced statistical methods were applied in the study.

RESULTS

Participants' Demographic Factors
The study involved 500 participants, with 74.80% attending Bo Government Hospital, an urban healthcare facility, and 25.20% attending Tikonko Maternity Home in a rural setting (**Table 1**). **Table 2** depicts that the mean age of pregnant women was 25 years (SD = 4.74), while that of newborn mothers was slightly lower at 24 years (**Table 3**), with both distributions skewed towards younger women. In **Table 4**, most newborns were aged between 29 days and 2 months (33.98%), followed by 28.64% aged 3 to 5 months.

Table 1: Participant Residency

Residency	*Health Facility*	*Frequency*	*Percent*
Urban	Bo Government Hospital	374	74.80
Rural	Tikonko Maternity Home	126	25.20
	Total	**500**	**100**

Table 2: Age (for pregnant women)

Category	*Findings*
Mean	24.826531
Standard Error	0.2765924
Median	25
Mode	25
Standard Deviation	4.7425721
Sample Variance	22.49199
Kurtosis	1.2373596
Skewness	0.829276
Range	29
Minimum	16
Maximum	45
Sum	7299
Count	294
Largest (1)	45
Smallest (1)	16
Confidence Level (95.0%)	0.5443597

Table 3: Age (for newborn women)

Category	*Findings*
Mean	24.067961
Standard Error	0.3021373
Median	23
Mode	20
Standard Deviation	4.3364865
Sample Variance	18.805115
Kurtosis	2.512482
Skewness	1.1848917
Range	28
Minimum	17
Maximum	45
Sum	4958
Count	206
Largest (1)	45
Smallest (1)	17
Confidence Level (95.0%)	0.595695

Table 4: Age (for newborn baby/child)

Age Category	*Frequency*	*Percent*
12 to 23 months old	19	9.22
Less than 24 hours old	1	0.49
1 to 28 days old	34	16.50
29 days to 2 months old	70	33.98
3 to 5 months old	59	28.64
6 to 11 months old	23	11.17
Total	**206**	**100**

The sex distribution of newborns was nearly equal, with 50.97% female and 49.03% male (**Table 5**). Regarding education, most participants had secondary education (53.60%), followed by primary education (28.40%), with smaller proportions having tertiary education (8.00%) or no formal education (10.00%) (**Table 6**). **Table 7** provides marital status, showing 46.60% of participants were married, while 31.60% were single. **Table 8** shows the population being split religiously between Islam (52.40%) and Christianity (47.40%).

Table 5: Sex for Newborn

Sex	*Frequency*	*Percent*
Female	105	50.97
Male	101	49.03
Total	**206**	**100**

Table 6: Participants' Education Level

Education Level	*Frequency*	*Percent*
Secondary education	268	53.60
Primary education	142	28.40
Tertiary education (college/university)	40	8.00
No formal education	50	10.00
Total	**500**	**100**

Table 7: Participants' Marital Status

Marital Status	*Frequency*	*Percent*
Married	233	46.60
Cohabitation	82	16.40
Single	158	31.60
Separated	19	3.80
Widowed	5	1.00
Divorced	3	0.60
Total	**500**	**100**

Table 8: Participants' Religions

Religion	*Frequency*	*Percent*
Islam	262	52.40
Christianity	237	47.40
Indigenous or Traditional Religions	1	0.20
Total	**500**	**100**

Professionally, participants were primarily students (34.80%) or housewives (22.60%), with 30.80% engaged in business (**Table 9**). The Mende tribe comprised 63.00% of participants, followed by 18.00% Temne (**Table 10**). Most families were monogamous (86.80%) (**Table 11**), with an average household size of 5.30 (**Table 12**). In **Table 13**, the mean monthly income was 335.03 New Leone (NLE), with significant income disparities indicated by high skewness (7.95). Finally, the mean number of pregnancies per woman was 1.83, with most women having experienced one or two pregnancies (**Table 14**).

Table 9: Participant Professions/Occupations

Participant Profession/Occupation	*Frequency*	*Percent*
Housewife	113	22.60
Student	174	34.80
Midwife	3	0.60
Business	154	30.80
Other Please specify	31	6.20
Educationist	2	0.40
Social Workers	3	0.60
Military	2	0.40
Agriculturist	13	2.60
Accountant	2	0.40
Paramilitary	1	0.20
Nurse	2	0.40
Total	**500**	**100**

Table 10: Participants' Tribes

Tribe	*Frequency*	*Percent*
Mende	315	63.00
Temne	90	18.00
Limba	40	8.00
Fula (or Fulani)	20	4.00
Sherbro	4	0.80
Kono	7	1.40
Mandingo	11	2.20
Kuranko	4	0.80
Others, please specify	1	0.20
Kru	1	0.20
Krio	2	0.40
Loko	3	0.60
Susu	1	0.20
Kissi	1	0.20
Total	**500**	**100**

Table 11: Participants' Family Type

Family Type	*Frequency*	*Percent*
Polygamous	66	13.2
Monogamous	434	86.8
Total	**500**	**100**

Table 12: Number of individuals living within the household

Parameters	*Findings*
Mean	5.304
Standard Error	0.182037
Median	4
Mode	4
Standard Deviation	4.070469
Sample Variance	16.56872
Kurtosis	41.85113
Skewness	5.041106
Range	50
Minimum	2
Maximum	52
Sum	2652
Count	500
Largest (1)	52
Smallest (1)	2
Confidence Level (95.0%)	0.357653

Table 13: Participant's monthly income in [New Leone (NLE)]

Category	*Findings*
Mean	335.034
Standard Error	29.89562
Median	150
Mode	100
Standard Deviation	668.4864
Sample Variance	446874
Kurtosis	95.5613
Skewness	7.946809
Range	10000
Minimum	0
Maximum	10000
Sum	167517
Count	500
Largest (1)	10000
Smallest (1)	0
Confidence Level (95.0%)	58.7368

Table 14: Number of pregnant experienced by the woman (primiparous and multiparous)

Parameter	*Findings*
Mean	1.834
Standard Error	0.046747
Median	2
Mode	1
Standard Deviation	1.045289
Sample Variance	1.092629
Kurtosis	4.581028
Skewness	1.83711
Range	6
Minimum	1
Maximum	7 and above
Sum	917
Count	500
Largest (1)	7 and above
Smallest (1)	1
Confidence Level (95.0%)	0.091845

Health-Seeking Behaviour During Pregnancy and After Childbirth

Table 15 shows that participants (100%) received antenatal care (ANC) services during pregnancy.

Many participants (71%) received ANC services from public health facilities, followed by community health centrees (24%), and a smaller percentage (4.6%) from private health facilities (**Table 16**).

A significant proportion of participants (44.4%) attended 3-4 ANC visits, while 41.2% attended 5 or more visits (**Table 17**).

The vast majority (99.03%) of participants gave birth in a healthcare facility, with only 0.97% delivering at home (**Table 18**).

Table 15: Antenatal Care (ANC) Services Received During Pregnancy

Response Ca tegory	*Frequency*	*Percent*
Yes	500	100
No	0	0.00
Total	**500**	**100**

Table 16: Site where ANC Services were primarily Received

Response Category	*Frequency*	*Percent*
Public health facility	355	71.00
Community health center	120	24.00
Private health facility	23	4.60
Other, Please Specify	2	0.40
Total	**500**	**100**

Table 17: Number of ANC visits received during most recent Pregnancy

Response Category	*Frequency*	*Percent*
5 or more visits	206	41.20
3-4 visits	222	44.40
1-2 visits	71	14.20
Not sure	1	0.20
Total	**500**	**100**

Table 18: Place of Childbirth

Response Category	*Frequency*	*Percent*
Home	2	0.97
Healthcare Facility	204	99.03
Total	**206**	**100**

Traditional Beliefs and Customs

In **Table 19**, 52.40% of respondents reported that their communities hold traditional beliefs related to pregnancy and childbirth. **Table 20** shows that 40.40% had personally participated in traditional practices, indicating strong cultural adherence. According to **Table 21**, the most common practices were attending traditional ceremonies (17.91%) and using herbal remedies (17.68%), followed by specific dietary restrictions (13.96%) and traditional birthing methods (12.95%).

As shown in **Table 22**, 37.60% of respondents believed traditional beliefs influenced their healthcare-seeking behavior, while 62.40% disagreed. In **Table 23**, 20.71% preferred traditional healers or midwives, 16.89% were influenced by traditional beliefs when choosing healthcare services, and 15.44% followed specific lifestyle or dietary customs.

However, **Table 24** reveals that 32.41% preferred evidence-based medical practices. Other reasons for rejecting traditional influences included unfamiliarity with traditional practices (18.63%) and a stronger trust in professional healthcare advice (14%). These findings suggest mixed but notable cultural influence on maternal care decisions.

Table 19: Present of traditional beliefs or customs in your community related to pregnancy, childbirth, or maternal health

Response Category	*Frequency*	*Percent*
Yes	238	52.40
No	262	47.60
Total	**500**	**100**

Table 20: Personal observation or participation in any traditional practices related to pregnancy and childbirth

Response Category	*Frequency*	*Percent*
Yes	202	40.40
No	298	59.60
Total	**500**	**100**

Table 21: Traditional practices related to pregnancy and childbirth observed or participated in

Response Category	*Multiple Response Frequency*	*Percent*
Participating in a traditional ceremony or ritual to bless the pregnancy or unborn child.	159	17.91
Using herbal remedies or traditional medicines recommended by elders or traditional healers during pregnancy or for childbirth.	157	17.68
Following specific dietary practices or restrictions based on cultural beliefs during pregnancy.	124	13.96
Engaging in traditional birthing practices, such as home births with the assistance of a midwife or doula.	115	12.95
Observing postpartum confinement or rest periods as dictated by cultural traditions.	75	8.45
Participating in naming ceremonies or other rituals for the newborn that are specific to my culture.	65	7.32
Adhering to traditional beliefs or superstitions regarding pregnancy and childbirth activities or behaviors.	43	4.84
Using traditional clothing or items during pregnancy or childbirth as dictated by cultural customs.	37	4.17
Following specific practices related to the announcement of pregnancy or the introduction of the newborn to the community.	31	3.49
Following specific traditions for naming or welcoming the newborn.	26	2.93
Engaging in specific physical activities or exercises that are traditional in my culture during pregnancy.	26	2.93
Practicing traditional methods of pain management during childbirth.	18	2.03
Observing cultural taboos or superstitions related to pregnancy and childbirth.	12	1.34
Total	**888**	**100**

Table 22: Believe that traditional beliefs and customs play a role in shaping your healthcare-seeking behavior during pregnancy and postnatal care

Response Category	*Frequency*	*Percent*
Yes	188	37.60
No	312	62.40
Total	**500**	**100**

Table 23: How traditional beliefs and customs shape your healthcare-seeking behavior during pregnancy and postnatal care

Response Category	*Multiple Response Frequency*	*Percent*
prefer consulting traditional healers or midwives for prenatal advice due to cultural beliefs.	157	20.71
Traditional beliefs influence my decision on the type of healthcare services I choose during pregnancy.	128	16.89
I follow specific dietary or lifestyle practices during pregnancy as advised by traditional customs.	117	15.44
Cultural customs dictate the timing or frequency of my healthcare visits.	108	14.25
Traditional customs and beliefs impact my willingness to seek certain medical interventions or treatments.	68	8.97
I seek healthcare providers who respect or incorporate traditional beliefs into their practice.	62	8.18
Cultural beliefs lead me to prioritize home remedies or natural approaches over conventional medical treatments.	45	5.94
Traditional beliefs affect my openness to discuss pregnancy-related issues with healthcare providers.	32	4.22
I engage in specific rituals or ceremonies believed to protect or benefit my pregnancy and seek healthcare that accommodates these practices.	24	3.17
My community's traditional beliefs influence my perception of the necessity and importance of prenatal care.	17	2.23
Total	**758**	**100**

Table 24: Why traditional beliefs and customs do not influence healthcare-seeking behavior during pregnancy and postnatal care

Response Category	*Multiple Response Frequency*	*Percentage*
I prioritize evidence-based medical practices over traditional beliefs for healthcare.	280	32.41
I am not familiar with or do not strongly identify with traditional practices in my community.	161	18.63
I believe in making healthcare decisions based on personal research and professional medical advice.	121	14
I have had negative experiences or outcomes with traditional practices in the past.	82	9.49
I find traditional beliefs and customs to be outdated or not applicable to modern healthcare.	78	9.03
My community or family does not strongly adhere to traditional practices.	57	6.6
I am influenced more by global or multicultural perspectives on healthcare.	46	5.32
I have concerns about the safety or efficacy of traditional practices during pregnancy.	27	3.13
There is a lack of traditional practices or customs related to pregnancy in my culture or community.	12	1.39
Total	**864**	**100**

Influence of Traditional Beliefs and Customs

The study reveals that 72% of respondents indicated no influence of traditional beliefs on their ANC or PNC decisions, while 16% reported negative and 12% positive influences (**Table 25**). Regarding the choice of healthcare providers, 41.4% acknowledged the role of traditional beliefs, whereas 58.6% were unaffected (**Table 26**). Among those influenced, 55.07% reported moderate influence, 26.57% a very strong influence, and 6.28% no influence (**Table 27**).

Table 25: How have traditional beliefs or customs influenced decisions regarding antenatal care (ANC) during recent pregnancy or recent postnatal care

Response Category	*Frequency*	*Percent*
They influenced me negatively	80	16.00
They had no influence	360	72.00
They influenced me positively	60	12.00
Total	**500**	**100**

Table 26: Have traditional beliefs or customs affected your choice of healthcare providers during pregnancy or after childbirth?

Response Category	*Frequency*	*Percent*
Yes	207	41.40
No	293	58.60
Total	**500**	**100**

Table 27: How traditional beliefs or customs influenced choice of healthcare providers

Response Category	*Frequency*	*Percent*
Slightly Influential	23	11.11
Very Influential	55	26.57
Extremely Influential	2	0.97
Moderately Influential	114	55.07
Not Influential at All	13	6.28
Total	**207**	**100**

Seeking Healthcare Against Traditional Beliefs

The study highlights a shift towards modern healthcare practices, with 65% of respondents seeking services during pregnancy or childbirth that went against traditional beliefs (**Table 28**), such as hospital births (27.72%), epidural use (15.75%), and cesarean sections (12.98%) (**Table 29**). However, 35% still adhered to traditional practices, reflecting resistance or limited access to modern care (**Table 28**). Key factors influencing this shift included access to evidence-based information (19.39%) and recommendations from medical professionals (18.19%), emphasizing the role of informed decision-making in adopting modern healthcare (**Table 30**).

Table 28: Seeking healthcare services during pregnancy or after childbirth against traditional beliefs or customs

Response Category	*Frequ ency*	*Percent*
Yes	325	65.00
No	175	35.00
Total	**500**	**100**

Table 29: Description of situations where seeking healthcare services during pregnancy or after childbirth went against traditional beliefs or customs

Response Category	*Multiple Response Frequency*	*Percentage*
I opted for a hospital birth over a traditional home birth due to medical advice or personal preference.	301	27.72
I chose to receive epidural anesthesia or other pain relief methods contrary to traditional practices of natural childbirth.	171	15.75
I pursued medical interventions like inductions or cesarean sections, despite cultural preferences for natural processes.	141	12.98
I decided to follow a diet or exercise plan recommended by healthcare professionals instead of traditional advice.	126	11.6
I engaged in prenatal screening and diagnostic tests that are not typically accepted in my cultural or traditional beliefs.	120	11.05
I sought mental health services or counseling for pregnancy-related issues, which is not commonly accepted in my tradition.	110	10.13
I participated in family planning or used contraception post-childbirth, which contradicts traditional beliefs in my community.	85	7.83
I challenged gender roles or expectations by involving my partner in ways not traditionally accepted during pregnancy or childbirth.	32	2.94
Total	**1086**	**100**

Table 30: Factors or influences encouraging healthcare services that contradict traditional practices

Response Category	*Multiple Response Frequency*	*Percentage*
Access to Evidence-Based Information	452	19.39
Medical Professional Recommendations	424	18.19
Safety and Health Outcomes	251	10.77
Research on Best Practices	235	10.08
Open Communication with Healthcare Providers	210	9.01
Informed Decision-Making	194	8.32
Community Health Programs	177	7.59
Peer Experiences	173	7.42
Cultural Sensitivity	154	6.61
Alignment with Personal Values	61	2.62
Total	**2331**	**100**

Integrating Cultural Sensitivity into Healthcare

The study reveals that 26.24% of respondents recommend training healthcare professionals in cultural competence, while 12.77% support integrating traditional practices where safe (**Table 31**). Notably, 74.4% advocate for bridging gaps between modern healthcare and traditional beliefs (T**able 32**). **Table 33** shows top suggestions: Community Health Worker Programs (19.52%) as the most preferred, followed by Cultural Competency Training (14.83%) to enhance understanding, and Community Engagement and Participatory Research (13.3%) to align health interventions with local values, ensuring cultural inclusivity and community acceptance.

Table 31: how can healthcare services be made more culturally sensitive and respectful of traditional beliefs and customs to address pregnancy and childbirth

Response Category	*Multiple Response Frequency*	*Percentage*
Provide training for healthcare professionals on cultural competence and understanding of diverse pregnancy and childbirth practices.	487	26.24
Incorporate traditional practices into healthcare services where possible and safe, in consultation with cultural experts.	237	12.77
Ensure that healthcare providers communicate effectively and respeafully with patients about their cultural needs and preferences.	218	11.75
Create spaces within healthcare facilities that are welcoming and respectful of cultural diversity and practices.	211	11.37
Develop and provide informational materials on pregnancy and childbirth that respect and acknowledge cultural variations.	161	8.67
Offer patients the option to consult with or include traditional healers or midwives as part of their healthcare team.	139	7.49
Facilitate community engagement initiatives to bet ter understand and integrate community-specific needs and values.	124	6.68
Implement flexible healthcare policies that allow for individualized care plans respecting patients' cultural backgrounds.	123	6.63
Encourage patient and family participation in decision-making processes to ensure culturally aligned healthcare choices.	114	6.14
Offer language support services to ensure clear communication for patients from diverse linguistic backgrounds.	42	2.26
Total	**1856**	**100**

Table 32: Acknowledgement of initiatives or educational programs to help bridge the gap between modern healthcare practices and traditional beliefs

Response Category	*Frequency*	*Percent*
Yes	372	74.40
No	128	25.60
Total	**500**	**100**

Table 33: Specific initiatives or educational programs that could help bridge the gap between modern healthcare practices and traditional beliefs

Response Category	*Multiple Response Frequency*	*Percentage*
Community Health Worker Programs	320	19.52
Cultural Competency Training for Healthcare Providers	243	14.83
Community Engagement and Participatory Research	218	13.3
Integrative Healthcare Services	197	12.02
Educational Workshops and Seminars	183	11.17
Traditional Healers Collaboration	163	9.95
Health Literacy Programs	125	7.63
Digital Health Education Platforms	75	4.58
School-Based Health Education	72	4.39
Policy Advocacy	43	2.61
Total	**1639**	**100**

Antenatal and Postnatal Care and the Presence of Skilled Birth Attendants

The study indicated that 94.6% reported being informed of the importance of being attended to by skilled birth attendants (**Table 34**), with 91% of respondents having a skilled birth attendant present during childbirth (**Table 35**).

Table 34: Provided information about the importance of having a skilled birth attendant during your ANC or PNC visits

Response Category	*Frequency*	*Percent*
Yes	473	94.60
No	27	5.40
Total	**500**	**100**

Table 35: Present of skilled birth attendant present during recent childbirth

Response Category	*Frequency*	*Percent*
Yes	455	91.00
No	45	9.00
Total	**500**	**100**

Influence of Skilled Birth Attendants

Skilled birth attendants influenced 93.85% of respondents to seek facility-based care (**Table 36**), promoting safe deliveries. However, financial constraints (30.21%) and transportation issues (27.08%) (**Table 37**) were key barriers for those without skilled attendants, highlighting challenges in accessing skilled care in resource-limited settings.

Table 36: How the presence of skilled birth attendants during childbirth influences decisions to give birth at a healthcare facility

Response Category	*Frequency*	*Percent*
It encouraged me to seek facility-based care	427	93.85
It did not influence my decision	28	6.15
Total	**455**	**100**

Table 37: Explanation for why pregnant mothers didn't have skilled birth attendants present during childbirth

Response Category	*Multiple Response Frequency*	*Percentage*
Financial constraints made it difficult to afford a skilled birth attendant or facility-based care.	29	30.21
I could not access a healthcare facility or skilled birth attendant due to distance or transportation issues.	26	27.08
I preferred a traditional home birth with a non-medical birth attendant, such as a traditional midwife or family member.	14	14.58
The onset of labor was too rapid or unexpected to reach a healthcare facility or summon a skilled attendant.	6	6.25
I had concerns or fears about receiving care in a healthcare facility or from a skilled birth attendant.	6	6.25
Cultural or family traditions favored unattended childbirth or the assistance of unskilled attendants.	6	6.25
Previous negative experiences with healthcare facilities or providers influenced my decision.	4	4.17
There was a lack of availability of skilled birth attendants or healthcare facilities in my area.	3	3.13
Personal preference for privacy or a familiar environment led me to choose childbirth without a skilled attendant.	2	2.08
Total	**96**	**100**

Antenatal Care (ANC), Postnatal Care (PNC), and Healthcare Levels

Most services were provided at secondary healthcare facilities (68.29%), followed by primary healthcare centres (28.86%) (**Table 38**). Minimal utilization of tertiary facilities (0.41%) and private facilities (2.44%) suggests potential barriers, such as accessibility issues or perceptions regarding the adequacy of lower-level facilities. These findings highlight the reliance on secondary and primary care levels and suggest a need to explore factors limiting the use of tertiary and private healthcare services.

Table 40: Provision of information on preventive measures and healthy practices during your ANC or PNC visits at the primary healthcare level

Response Category	*Frequency*	*Percent*
Yes	140	98.59
No	2	1.41
Total	**142**	**100**

Table 38: Level of healthcare facilities where primarily ANC or PNC services were received

Response Category	*Frequency*	*Percent*
Secondary healthcare facility (e.g., district hospital)	336	68.29
Primary healthcare center	142	28.86
Other (please specify) (Private facility)	12	2.44
Tertiary healthcare facility (e.g., regional or national hospital)	2	0.41
Total	**492**	**100**

Influence of Primary Healthcare in Establishing and Maintaining Health

Primary healthcare services significantly influenced health-seeking behaviour, with 59.16% of respondents finding them "very influential" and 33.80% "moderately influential" during pregnancy and childbirth (**Table 39**). Nearly all women (98.59%) reported receiving preventive information during ANC or PNC visits (**Table 40**). Key preventive measures provided included regular check-ups (13%), rest and sleep (10.48%), exercise (10.38%), nutrition (10.28%), and avoiding harmful substances (8.54%) (**Table 41**). These services and information play a vital role in promoting healthy practices and improving maternal and neonatal health outcomes.

Table 39: How primary healthcare services received during your pregnancy, childbirth, or newborn care influence healthcare-seeking behavior in establishing and maintaining a healthy physical and mental status

Response Category	*Frequency*	*Percent*
Very Influential	84	59.16
Extremely Influential	3	2.11
Moderately Influential	48	33.80
Slightly Influential	2	1.41
Not Influential at All	5	3.52
Total	**142**	**100**

Table 40: Provision of information on preventive measures and healthy practices during your ANC or PNC visits at the primary healthcare level

Response Category	*Frequency*	*Percent*
Yes	140	98.59
No	2	1.41
Total	**142**	**100**

Table 41: Key information on preventive measures and healthy practices given during ANC or PNC visits at the primary health level

Response Category	*Multiple Response Frequency*	*Percentage*
Regular Check-ups	134	13
Rest and Sleep	108	10.48
Exercise	107	10.38
Nutrition	106	10.28
Avoid Harmful Substances	88	8.54
Hydration	77	7.47
Immunizations	59	5.72
Regular Monitoring of Blood Pressure and Weight	55	5.33
Emotional Well-being	54	5.24
Education on Pregnancy and Childbirth	51	4.95
Screening for Infections	48	4.66
Screening for Gestational Diabetes	42	4.07
Birth Plan	39	3.78
Preparation for Breastfeeding	36	3.49
Partner Involvement	27	2.61
Total	**1031**	**100**

Influence of Secondary Healthcare Services in Dealing with Degeneration from a Healthy State

Table 42 highlights that 81.25% of participants experienced health issues during pregnancy, childbirth, or newborn care that required care beyond primary healthcare, indicating significant reliance on secondary-level services.

Table 43 shows that the availability of secondary healthcare was moderately (53.58%) to very influential (28.27%) for most respondents when deciding to seek care for pregnancy-related health complications.

Table 42: Experiencing health issues or complications during your recent pregnancy, childbirth, or newborn care that required medical care beyond primary healthcare

Response Category	*Frequency*	*Percent*
Yes	273	81.25
No	63	18.75
Total	**336**	**100**

Table 43: How availability of secondary healthcare facilities (e.g., district hospital) influence decisions to seek medical care when dealing with health issues during pregnancy, childbirth, or newborn care

Response Category	*Frequency*	*Percent*
Moderately Influential	180	53.58
Very Influential	95	28.27
Extremely Influential	7	2.08
Slightly Influential	53	15.77
Not Influential at All	1	0.30
Total	**336**	**100**

Integrated Approaches and Suggestions

Table 44 presents strategies for aligning healthcare levels. Participants emphasized improved communication (18.51%), enhanced training (14.83%), and standardized referral pathways (11.94%) as top priorities for better integration across primary, secondary, and tertiary care.

Table 45 identifies decision-making influences for ANC/PNC use. Most participants (77.19%) were motivated by protective factors, while 21.59% cited alleviating factors, and only 1.22% mentioned aggravating factors. These findings suggest that preventive health awareness and proactive care promotion play a critical role in encouraging timely maternal healthcare utilization.

Table 44: how can the different levels of healthcare (Primary, Secondary, and Tertiary) be better aligned (work together) to support pregnant women and newborns

Response Category	*Multiple Response Frequency*	*Percentage*
Improved Communication	468	18.51
Enhanced Training Programs	375	14.83
Standardized Referral Pathways	302	11.94
Community Engagement	276	10.91
Utilization of Technology	272	10.76
Quality Assurance Measures	206	8.15
Feedback Mechanisms	180	7.12
Streamlined Access to Resources	179	7.08
Continuous Monitoring and Evaluation	159	6.29
Multi-disciplinary Teams	112	4.41
Total	**2529**	**100**

Table 45: Factors influencing Maternal decision to seek ANC or PNC services

Response Category	*Frequency*	*Percent*
Protective factors (e.g., preventive care)	379	77.19
Alleviating factors (e.g., addressing existing health issues)	106	21.59
Aggravating factors (e.g., complications or risks)	6	1.22
Total	**491**	**100**

DISCUSSION

Participant Residency

The study included 500 participants, with 74.80% attending Bo Government Hospital (urban) and 25.20% attending Tikonko Maternity Home (rural). This disparity reflects higher urban healthcare utilization due to better infrastructure, accessibility, and service availability [12]. These findings emphasize the need for targeted interventions to improve rural healthcare access and maternal health outcomes.

Age Distribution for Pregnant Women, Newborn Women, and Newborns

Pregnant women's mean age was 25 years (skewness = 0.83), while newborn mothers had a mean age of 24 years (skewness = 1.18), reflecting a younger demographic typical of Sub-Saharan Africa [13]. Most newborns were 29 days to 2 months old (33.98%), followed by 3 to 5 months old (28.64%), highlighting a focus on early postnatal care during the critical neonatal phase [14].

Education Level

Most participants had secondary education (53.60%), followed by primary (28.40%), tertiary (8.00%), and no formal education (10.00%). Higher education levels correlate with better maternal health-seeking behaviour, underscoring the importance of education in improving healthcare outcomes [15].

Marital Status and Religion

Nearly half of the participants were married (46.60%), with 31.60% single. Married women benefit from greater support and resources, improving maternal health-seeking behaviour [16]. Religious composition was balanced between Islam (52.40%) and Christianity (47.40%), suggesting interventions should engage religious leaders to influence health-seeking behaviours positively [17].

Occupation, Tribe, and Family Type

Students (34.80%), housewives (22.60%), and businesswomen (30.80%) dominated the sample, reflecting diverse socio-economic needs [18]. The Mende tribe comprised 63.00% of participants, followed by Temne (18.00%), highlighting the cultural context's role in shaping maternal health behaviours [19]. Most families were monogamous (86.80%), with polygamous families (13.20%) potentially facing more resource constraints [5].

Household Size, Income, and Pregnancy Experience

The mean household size was 5.30, and the mean monthly income was 335.03 NLE, with significant financial disparities affecting healthcare access [20,21]. Participants had a mean of 2 pregnancies, emphasizing the need for maternal education and services across all parity levels [22].

Health-Seeking Behaviour During Pregnancy and After Childbirth

There was a 100% utilization rate of antenatal care (ANC) services among participants, demonstrating the success of maternal health outreach programs in the region. Most participants (71%) accessed ANC through public healthcare facilities, reflecting their affordability and accessibility in low-resource settings. A significant proportion of women attended the recommended 3 to 4 ANC visits (44.4%), with 41.2% exceeding this, aligning with World Health Organization guidelines for early monitoring and intervention during pregnancy [23,24].

Nearly all participants (99.03%) delivered in healthcare facilities, showcasing a high institutional delivery rate, critical for reducing maternal and neonatal mortality through skilled birth attendance and emergency obstetric care [25]. The study highlights the effectiveness of

government-supported maternal health services in promoting ANC and facility-based deliveries, which are pivotal for improved maternal and neonatal health outcomes.

Traditional Beliefs and Customs

Traditional beliefs and customs influenced pregnancy and childbirth by 52.40%, emphasizing their continued relevance, particularly in rural areas. Approximately 40.40% observed or participated in traditional practices such as ceremonies (17.91%), herbal remedies (17.68%), dietary restrictions (13.96%), and traditional birthing methods (12.95%). While these practices are culturally significant, challenges arise when they conflict with evidence-based medical care, highlighting the importance of culturally sensitive healthcare approaches [7,11,26,27].

Traditional beliefs shaped healthcare-seeking behaviour for 37.60% of respondents, primarily influencing preferences for traditional healers or midwives (20.71%) and specific lifestyle practices (15.44%). However, 62.40% cited modern healthcare accessibility, education, and awareness as stronger determinants of their health decisions. For 65% of respondents, healthcare choices sometimes contradicted traditional customs, indicating a shift towards evidence-based practices driven by improved healthcare access and awareness [28,29,30,34].

While 72% of respondents indicated that traditional beliefs did not affect their antenatal or postnatal care decisions, 16% experienced negative influences, potentially discouraging service utilization. To address this, integrating trained traditional birth attendants into formal healthcare systems could bridge the gap between traditional and modern practices, ensuring safety and cultural sensitivity [31-33].

These findings highlight the dual importance of respecting cultural norms and promoting education on modern healthcare benefits. A WHO-endorsed culturally appropriate healthcare model that integrates traditional practices with evidence-based care can help improve maternal and newborn health outcomes in resource-limited settings [35].

Integrating Cultural Sensitivity into Healthcare

The study highlights the importance of culturally sensitive care, with 26.24% of respondents advocating for healthcare provider training in cultural competence to improve outcomes, satisfaction, and trust [36]. Additionally, 74.4% supported initiatives to bridge modern healthcare and traditional beliefs, promoting culturally integrated approaches that enhance maternal and newborn health [6,8]. These findings underscore the need for evidence-based strategies that respect cultural practices while fostering dialogue between providers and communities to improve healthcare acceptance and utilization.

Antenatal and Postnatal Care and the Presence of Skilled Birth Attendants

Ninety-seven percent of women accessed antenatal/postnatal care (ANC/PNC), and 91% had skilled birth attendants during childbirth, emphasizing the importance of skilled professionals in improving maternal and neonatal outcomes [9]. Skilled attendants also encouraged 93.85% of women to seek facility-based care, promoting safer childbirth practices [38]. However, financial constraints (30.21%) and transportation issues (27.08%) hindered access, reflecting systemic barriers in low-resource settings that require targeted interventions to enhance accessibility [39]. These findings align with global recommendations advocating for skilled birth attendance to reduce maternal and neonatal

mortality [31].

Healthcare for Newborns

96.12% of respondents sought healthcare for newborns, with 96.46% prioritizing skilled providers. While awareness of neonatal care is high, geographic and logistical barriers persist. Expanding neonatal services, especially in rural areas, is critical to improving survival rates and addressing preventable neonatal complications [40].

Encouraging Skilled Birth Attendance

Encouraging skilled birth attendance involves strategies such as training programs (22.6%), mandatory skilled attendance policies (13.91%), and improving healthcare access (12.64%) [10]. Community education, government-subsidized services, and mobile health clinics also address disparities, aligning with successful global practices in low-resource settings [41,42].

Antenatal Care (ANC) and Postnatal Care (PNC) Services

Antenatal and postnatal care (ANC/PNC) services were utilized by 98.40% of women, underscoring high engagement with maternal healthcare, critical for monitoring health and addressing complications [43]. Secondary healthcare facilities were preferred (68.29%) over primary (28.86%) and tertiary (0.41%) facilities, reflecting perceptions of balanced accessibility and quality [44-46]. The low use of tertiary facilities highlights barriers such as cost, distance, and awareness, necessitating targeted interventions to improve access to specialized care.

CONCLUSION

The urban-rural disparity emphasizes the need for improved rural healthcare infrastructure.

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Framework Influencing Healthcare Services Utilization: Impact on Maternal Health-Seeking Behaviour and Key Factors

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ABSTRACT

Introduction: Maternal and neonatal mortality remain critical challenges in Sub-Saharan Africa, with Sierra Leone among the worst-affected countries. Barriers such as socio-cultural norms, financial constraints, and limited infrastructure hinder healthcare-seeking behaviors among pregnant women and mothers in rural areas. This study examines the influence of protective, alleviating, and aggravating factors on healthcare-seeking behavior among pregnant women and newborn mothers in Bo District, Sierra Leone.

Materials and Methods: A mixed-methods study was conducted among 500 participants, including 294 pregnant women and 206 newborn mothers, at Bo Government Hospital and Tikonko Village Health Center. Data were collected via structured questionnaires and analyzed using Chi-Square, logistic regression, and Structural Equation Modeling (SEM) to assess relationships among influencing factors.

Results: Protective factors, including health education and preventive care awareness, significantly influenced healthcare-seeking behavior, reported by 97.2% of participants. Alleviating factors such as access to healthcare (20.29%) and financial support (15.1%) promoted high antenatal care utilization (100%) and facility-based delivery (99.03%). Aggravating factors like cultural barriers and perceived risks negatively impacted care-seeking, with SEM showing a significant indirect effect ($\beta = -0.45$, $p < 0.001$).

Conclusion: Health education, accessibility, and financial support are critical for improving maternal and neonatal health outcomes. Addressing socio-cultural barriers and enhancing risk communication are essential for further progress. Policymakers should prioritize integrated strategies to reduce maternal and neonatal mortality in Sierra Leone.

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INTRODUCTION

Maternal and neonatal health outcomes in Sub-Saharan Africa continue to be a significant public health challenge, with the region accounting for the highest rates of maternal and neonatal mortality globally. Sierra Leone faces one of the highest maternal mortality ratios in the world, with 1,360 deaths per 100,000 live births [1-3]. Despite the efforts of international and national health interventions aimed at improving maternal healthcare, many women in Sierra Leone, especially those in rural areas, still face significant barriers to accessing healthcare services during pregnancy and after childbirth [4-6]. Healthcare-seeking Behaviours among pregnant women and newborn mothers are influenced by a complex interplay of factors, including protective, alleviating, and aggravating influences.

Protective factors such as health education, community support, and access to healthcare information play a crucial role in motivating women to seek care. On the other hand, alleviating factors, including access to healthcare facilities and financial support, help reduce some of the systemic barriers' women face. However, aggravating factors such as cultural practices, geographic isolation, and socio-economic constraints can significantly hinder the timely utilization of maternal health services [7-9].

This study focuses on Bo District in Sierra Leone, where traditional practices, combined with socio-economic and infrastructural challenges, present unique obstacles to healthcare access. By exploring the role of protective, alleviating, and aggravating factors in influencing healthcare-seeking Behaviour, this research provides insights into the determinants of maternal and neonatal healthcare utilization in the district. These insights are critical to informing policy interventions and strategies that could enhance maternal and neonatal health outcomes.

Study Objectives

The specific objectives of this research are:

1. To assess the influence of protective factors, such as health education, community support, and access to preventive healthcare services, on the healthcare-seeking Behaviour of pregnant women and newborn mothers.
2. To examine the role of alleviating factors, including improved access to healthcare facilities, financial support, and community health interventions, in promoting the utilization of healthcare services among pregnant women and newborn mothers.
3. To analyze the impact of aggravating factors, such as socio-cultural barriers, economic constraints, and perceived risks, on healthcare-seeking Behaviours during pregnancy and postnatal care.

METHODS

Study Design

This research applied a mixed-methods approach that integrated quantitative and qualitative data collection techniques and investigated the influence of protective, alleviating, and aggravating factors on healthcare-seeking Behaviours among pregnant women and newborn mothers.

Study Setting

The study was conducted in Bo District, Sierra Leone, at two key healthcare facilities namely Bo Government Hospital (an urban setting), and Tikonko Village Health Center (a rural setting). Participants

Using Cochran's formula, the study initially calculated a sample size of 370 participants. To strengthen the study's rigor, this number was increased by 26%, adding 130 participants, resulting in a total sample size of 500. Among the participants, 294 were pregnant women, and 206 were mothers of newborns. This increase accounted for potential non-responses, enhanced population representativeness, and minimized sampling errors. The expanded sample ensured sufficient statistical power and captured a wider range of population variability, thereby improving the generalizability of the findings. This adjustment adhered to ethical research standards, particularly for sensitive groups like pregnant women and newborn mothers, ensuring actionable and meaningful outcomes. A simple random sampling method was applied to ensure that diverse socio-demographic characteristics such as age, education level, income, marital status, and religious affiliations were represented. Inclusion criteria required participants to be

either a pregnant woman or a mother of a newborn, reside in Bo District, and have accessed healthcare services at either Bo Government Hospital or Tikonko Village Health Center.

Data Collection

Data collection took place from March to May 2024 and involved the use of structured questionnaires administered via ONA, a mobile data collection application. ONA helped in getting real-time data and minimizing data collection errors. Ten trained data enumerators administered the questionnaires, ensuring consistency and reliability in data collection. Additionally, participants were provided informed consent before taking part in the study, ensuring ethical compliance. Ethical approval from obtained from the Njala University Institutional Review Board.

Data Analysis

Statistical analyses were carried out using SPSS and AMOS for descriptive and inferential statistics. The following analytical techniques were employed: Chi-Square Test of Independence, Logistic Regression, ANOVA, and Structural Equation Modeling (SEM).

Additionally, qualitative data from open-ended questions were analyzed using thematic analysis to provide deeper insights into the socio-cultural and contextual factors influencing healthcare utilization.

RESULTS

Participant Demographics

The study included 500 participants, with a majority (74.80%) attending Bo Government Hospital, an urban healthcare facility, while 25.20% attended Tikonko Maternity Home, located in a rural area.

The mean age of pregnant women was 25 years, slightly higher than that of newborn mothers at 24 years, reflecting a predominance of younger women. Most newborns (33.98%) were aged between 29 days and 2 months, followed by 28.64% aged 3 to 5 months.

The sex distribution of newborns was nearly equal, with 50.97% female and 49.03% male.

Participants predominantly had secondary education (53.60%), followed by primary education (28.40%). A smaller portion had tertiary education (8.00%), and 10.00% had no formal education.

Many participants were married (46.60%), with a significant number being single (31.60%).

The religious composition was nearly evenly split between Islam (52.40%) and Christianity (47.40%), reflecting the general religious demographics of the region.

A significant portion of participants were students (34.80%) or housewives (22.60%), with 30.80% engaged in business.

The Mende tribe constituted the majority (63.00%), followed by the Temne (18.00%). Most families were monogamous (86.8%), with only 13.2% being polygamous. The mean household size was 5.30, with a wide range from 2 to 52 individuals. The mean monthly income was 335.03 New Leone (NLE), with significant skewness (7.95), indicating that most households earn below this average, with a few high outliers.

The mean number of pregnancies was 2, with a skewness of 1.84, indicating that most women had experienced one or two pregnancies.

Health-Seeking Behaviour During Pregnancy and After Childbirth

All participants (100%) received ANC services during pregnancy, highlighting the success of maternal health outreach and awareness programs. Many participants (71%) received ANC services from public health facilities, followed by community health centers (24%), and a smaller percentage (4.6%) from private health facilities.

A significant proportion of participants attended 3-4 ANC visits (44.4%), while 41.2% attended 5 or more visits.

The vast majority (99.03%) of participants gave birth in a healthcare facility, with only 0.97% delivering at home.

Influence of Protective Factors

A significant proportion of respondents (97.20%) reported receiving information on preventive measures. This knowledge was found to be "moderately influential" (43.80%) or "very influential" (38.80%) in shaping maternal health-seeking Behaviour.

Influence of Alleviating Factors

The most influential alleviating factors identified were improved access to healthcare facilities (20.29%), increased awareness through education and community outreach programs (17.14%), and reduced financial barriers through affordable or free services (15.1%).

Influence of Protective Factors on Maternal Health Choices:

- **Accessible Healthcare Facilities:** Many respondents (35.60%) rated accessible healthcare facilities as "Very Influential" in their healthcare decisions, with 25.40% finding them "Extremely Influential."
- **Affordable Healthcare:** A significant portion of respondents (33.60%) found affordable healthcare to be "Very Influential," with 27.80% rating it as "Moderately Influential."
- **Health Education:** 32.80% of respondents considered health education "Very Influential," while 24.40% considered it "Moderately Influential."

Influence of Aggravating Factors

A high percentage (95.20%) of respondents were informed of risks or complications during pregnancy, with this knowledge being "very influential" (41.40%) or "moderately influential" (41.20%) in shaping healthcare-seeking Behaviour.

Healthcare-Seeking Behaviour for Newborns

Most respondents (96.12%) sought healthcare services for their newborns. The decision to seek healthcare services for newborns was primarily influenced by protective factors (81.31%), suggesting a strong commitment to preventive care.

Integrated Approaches and Suggestions

The following strategies were recommended for addressing Protective, Alleviating, and Aggravating factors:

- Community-Based Education (13.71%)
- Maternal and Child Health Clinics (10.14%)
- Early Antenatal Care (9.37%)

Antenatal Care (ANC) and Predisposing Level Factors

For the utilization of ANC or PNC Services, a high percentage (97.40%) of respondents received ANC or PNC services, with knowledge and awareness (82.96%) being the most influential predisposing factors.

Enabling Level Factors

The study revealed the presence of Enabling Factors. A significant portion of respondents (71.20%) reported enabling factors that facilitated access to healthcare, with financial stability (9.88%), health insurance coverage (9.7%), and transportation availability (8.62%) being the most cited enabling factors. These factors positively influenced healthcare-seeking Behaviour in 40.45% of respondents.

Illness Level Factors

Many respondents (68.20%) experienced health issues requiring care during or after pregnancy. These illness-level factors were "moderately influential" (45.16%) or "very influential" (30.21%) in the decision to seek healthcare, underscoring the impact of health complications on healthcare-seeking Behaviour.

Integrated Approaches for Predisposing, Enabling, and Illness Factors

Strategies for addressing Predisposing Factors were increasing access to prenatal education programs (25.91%), and community outreach to promote early antenatal are (24.12%).

The participants acknowledged the following strategies to address Enabling Factors:

- Enhancement of Transportation Options (24.6%)
- Expanding Access to Affordable Maternal Healthcare Services (24.21%)
- Establishing Community-Based Support for Pregnant Women and Newborn Mothers (22.76%).

The following strategies were reported by participants to address Illness Factors:

- Strengthening Maternal and Child Health Surveillance Systems (27.55%)
- Improving Access to Prenatal Screening and Diagnostic Services (23.08%)
- Increasing Availability of Skilled Birth Attendants and Emergency Obstetric Care Facilities (21.69%).

DISCUSSION

Participant Demographics

This study provides valuable insights into the characteristics of the study population and their influence on healthcare-seeking Behaviour. A significant portion of participants (74.80%) sought services from Bo Government Hospital, an urban facility, highlighting the preference for urban healthcare settings. This aligns with studies that indicate urban facilities are favored due to better accessibility, infrastructure, and resources [10,11,13]. The finding suggests that rural areas require infrastructural and quality improvements to balance healthcare access.

The mean age of pregnant women was 25 years (SD = 4.74), with newborn mothers slightly younger at 24 years (SD = 4.74). These ages reflect early childbearing trends prevalent in Sub-Saharan Africa [14]. The sex ratio of newborns, with 50.97% female and 49.03% male, mirrors the natural distribution and indicates a representative sample [10].

Education levels also play a crucial role, with 53.60% having secondary education, 28.40% primary, and 8.00% tertiary education. Education strongly correlates with health literacy and the likelihood of seeking healthcare services [15]. However, 10% had no formal education, highlighting disparities that may restrict healthcare access for this subset.

Marital status showed nearly half (46.60%) of participants were married, while 31.60% were single. Marriage often provides emotional and financial support, facilitating better healthcare

access [16]. Conversely, single women may face more significant barriers due to limited resources or support networks.

Socio-economic factors were evident, with a mean household size of 5.30 and a mean monthly income of 335.03 NLE, indicating financial constraints. The skewed income distribution suggests that poverty is a barrier to healthcare, especially for frequent antenatal visits or facility-based deliveries [11]. On average, women had 2 pregnancies, and multiparous women were more familiar with the healthcare system, increasing their likelihood of seeking care [17].

Health-Seeking Behaviour During Pregnancy and After Childbirth

The findings reveal a 100% antenatal care (ANC) utilization rate, demonstrating the effectiveness of maternal health outreach programs in Bo District. Public healthcare facilities were the most utilized (71%), followed by community health centers (24%) and private facilities (4.6%). The preference for public facilities reflects their affordability, though the low use of private facilities suggests cost is a limiting factor [18].

Most participants (44.4%) attended 3–4 ANC visits, while 41.2% attended five or more, meeting the World Health Organization (WHO) recommendations of at least four visits [12]. This high adherence underscores the success of health education campaigns in raising awareness about the importance of regular ANC visits.

Facility-based deliveries were reported at 99.03%, a positive outcome linked to policies promoting skilled birth attendance and emergency obstetric care. However, the 0.97% who delivered at home highlight persistent barriers in remote or rural areas where healthcare access is limited [19].

Influence of Protective, Alleviating, and Aggravating Factors

Protective factors, such as health education, significantly influenced healthcare-seeking Behaviour, with 97.20% of participants reporting awareness of preventive measures. Educational interventions emerged as key motivators, with 43.80% finding this information "moderately influential" and 38.80% finding it "very influential." These findings align with global evidence that health education enhances maternal healthcare utilization [20,21].

Alleviating factors, including improved healthcare access (20.29%), education (17.14%), and reduced financial barriers (15.1%), were pivotal in promoting healthcare-seeking. Accessibility to healthcare facilities was rated "very influential" by 35.60% of respondents, with affordability cited as a key enabler [22]. The findings underscore the importance of reducing logistical and financial constraints to improve utilization, particularly in rural areas.

Aggravating factors, such as awareness of risks and complications, were reported by 95.20% of participants, with 41.40% finding this information "very influential." Risk awareness motivates early and frequent care-seeking, particularly in emergencies [23,24]. However, financial and logistical barriers may prevent women from acting on this knowledge, necessitating integrated approaches that combine risk communication with accessible and affordable services.

Comparison with Existing Literature

These findings are consistent with research from Sub-Saharan Africa, which identifies education, access, and affordability as key determinants of maternal healthcare utilization [11,25]. Educational

campaigns in low-income settings have significantly improved ANC attendance and facility-based deliveries [23,26]. The high facility-based delivery rate observed in this study aligns with other countries in the region that have implemented successful maternal health outreach programs [26].

However, this study adds nuance by highlighting the role of aggravating factors, particularly risk awareness. While previous studies emphasize the importance of risk communication, this research shows that awareness alone cannot overcome financial and logistical barriers [28,29].

CONCLUSION

This study demonstrates the significant influence of **protective, alleviating, and aggravating factors** on healthcare-seeking Behaviours among pregnant women and newborn mothers in Bo District, Sierra Leone. **Protective factors** like awareness of preventive measures (97.2%) strongly contributed to positive healthcare-seeking, as evidenced by high antenatal care (ANC) utilization (100%) and facility-based delivery rates (99.03%). **Alleviating factors**, such as improved access to healthcare facilities (20.29%) and financial support (15.1%), played critical roles in facilitating healthcare access. On the other hand, **aggravating factors**, particularly risk awareness, negatively affected care-seeking, suggesting that fear of complications and other barriers hinder timely healthcare utilization. These findings highlight the need for **multi-stakeholder interventions** to address protective and aggravating factors to improve maternal and newborn health outcomes.

RECOMMENDATIONS WITH STAKEHOLDER RESPONSIBILITIES

1. **Enhance Health Education Campaigns:**
   - **Local Community and Local Leaders:** Organize community gatherings and initiatives to raise awareness about the importance of antenatal care (ANC) and facility-based deliveries.
   - **Healthcare Providers and Health Facilities:** Deliver consistent health education during ANC visits, focusing on preventive measures and available services.
   - **District Health Authority:** Implement community outreach programs targeting remote and underserved areas to improve maternal health education.
   - **Ministry of Health & National Government:** Launch nationwide media campaigns and public health messaging to promote healthcare-seeking Behaviours.
2. **Improve Healthcare Accessibility:**
   - **Local Community and Local Leaders:** Advocate for improved road infrastructure and transport services to connect remote areas to health facilities.
   - **District Health Authority:** Establish additional health posts and mobile health services in underserved areas.
   - **Ministry of Health and National Government:** Invest in rural healthcare infrastructure development to reduce geographic disparities in service delivery.
3. **Alleviate Financial Barriers:**
   - **Local Leaders:** Facilitate the formation of community savings groups or microfinance initiatives to support women in accessing maternal healthcare.
   - **District Health Authority:** Collaborate with local stakeholders to develop community-based health insurance schemes and financial support mechanisms.
   - **Ministry of Health:** Introduce or expand subsidies and voucher programs

for maternal health services to reduce out-of-pocket expenses for low-income households.

- **National Government:** Support comprehensive national health insurance programs and allocate sufficient budgetary resources to make maternal healthcare affordable.

4. **Strengthen Risk Communication:**
   - **Healthcare Providers:** Train providers to communicate clearly and empathetically about pregnancy risks and complications to empower women.
   - **Training Institutions:** Incorporate effective risk communication into healthcare training curricula to equip future providers with these skills.
   - **District Health Authority:** Design district-level strategies and outreach programs to address fears related to pregnancy complications and promote healthcare options.
   - **Ministry of Health:** Establish national guidelines and ensure providers are trained in effective risk communication techniques.
5. **Address Cultural Barriers:**
   - **Local Leaders:** Work with traditional leaders and cultural influencers to address harmful beliefs and practices while respecting cultural norms.
   - **Healthcare Providers:** Collaborate with traditional birth attendants (TBAs) and community health workers to integrate traditional practices with modern maternal healthcare.
   - **District Health Authority:** Develop culturally sensitive maternal health programs that involve traditional healers and community leaders.
   - **Ministry of Health:** Create a national framework to integrate traditional practices with modern healthcare, ensuring that cultural beliefs are respected while prioritizing maternal health outcomes.

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Pattern of Microscopic Urine Examination in A Single Nigerian Centre: A Retrospective Cross-sectional Study

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ABSTRACT

Introduction: Urine microscopy is a relevant, inexpensive and non-invasive investigation. However, its usefulness depends on the availability of the appropriate equipment and expertise often lacking in low-resource countries. This study aimed to describe the pattern of urinary abnormalities, the frequency of urinary tract infections, the pattern of microorganisms causing UTI, and the antibiotic sensitivities.

Materials and Methods: A retrospective cross-sectional study conducted in a tertiary institution in southern Nigeria, using the urine microscopy results of all patients tested over a 6month period. Data were collated using Microsoft Excel and analyzed on IBM SPSS version 20.

Results: The frequency of haematuria and pyuria were 19% and 52.3% respectively. The frequency of pyuria was higher in females than males (64% vs. 42%, P=0.003 and X^2=9.09). There were 60.7% of males with haematuria and 32.4% of females with haematuria (X^2=5.16 and P= 0.023). Casts were seen in only 2.7% and crystals in 6.5% of the sample. Of the 152 patients, 47.1% had significant cultures and the most common organism grown was E. coli (40%). UTI was frequent in males compared to females (53.6% vs 41.7%, p=0.188). E.coli was most sensitive to Amoxicillin/Clavulanic acid (48.3%).

Conclusion: The frequency of haematuria and pyuria is higher compared to casts and crystalluria. Haematuria was significantly commoner in males while more females had pyuria. E.coli was the most frequent organism implicated. There is a need for phase-contrast microscopes in Nigerian hospitals to improve diagnosis of urine sediments.

Keywords: urine microscopy, haematuria, UTI

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INTRODUCTION

The urine has been one of the most readily assessed bodily fluids with diagnostic significance for centuries, but its usefulness in clinical practice depends on the availability of equipment and skill. In 400 BC Hippocrates conducted the first urine microscopy,[1,2] after that, in the 17th century following the invention of the microscope, Peirsc and Van Leeuenhoek revolutionized microscopic urine examination. Since then, it has remained one of the most relevant, inexpensive and non-invasive investigations used for centuries to diagnose medical conditions from infections to drug intoxication. Other technological advances in urine examination include the dipsticks, phase contrast microscopes, immunofluorescences, Polymerase Chain Reaction amongst others. [3,4]

The parameters assessed in the urine ranges from cells (red blood cells, white blood cells, pus cells, epithelial cells) casts, crystals, microorganisms, and the sensitivity of bacteria growth to antibiotics. Haematuria is one of the most prevalent microscopic urinary abnormalities in practice, and it is classified as glomerular and non-glomerular in origin based on the presence or absence of dysmorphic red cells [3,4] Leucocyturia usually depicts an infective process; however, leucocytes can be found in interstitial nephritis. Presence of leucocytes and erythrocytes is suspicious of Glomerulonephritis(GN)[3,4] Renal tubular epithelial cells are derived from exfoliation of the tubular epithelium, and transitional epithelial cells signify exfoliation of the uroepithelium,[5,6] that is seen in acute tubular necrosis and cystitis respectively.

Casts are cylindrical structures of variable length that form in the distal and collecting ducts of the kidney. The Tamm Horsfall glycoprotein secreted from the thick ascending loop of Henle makes up the matrix.[7,8] The commonest casts include the hyaline cast, granular cast, red cell cast and white cell cast, and each may be indicative of a specific disease process. Crystals are a sign of urine supersaturation with substances derived from metabolism, inherited diseases or drugs, [9]they are classified into four categories, namely, common, pathological, drugs, and other crystals. The commonest crystals seen on urine examination are the calcium oxalate. [10,11] Organisms isolated in urine vary according to age, sex, method of urine collection, [12] and sexual practices amongst others; however, bacteria are the most frequent organisms to be isolated with E.coli being the commonest bacteria. [13,14]

The pattern of urine abnormalities varies from region to region, based on demographic characteristics, socio-cultural/behavioural factors, health status, and pre-existing co-morbidities. However, the wrong technique of urine collection and examination may significantly mask the real epidemiological picture. Health institutions in resource-poor areas are often not equipped for reliable renal histopathologic services; therefore, microscopic urine exam is vital. This retrospective hospital-based cross-sectional study was aimed at describing the pattern of urine abnormalities seen in a single tertiary institution, specifically the frequency of urine abnormalities, culture patterns and the sensitivity.

MATERIALS AND METHODS

Study design and Area

This study was a retrospective cross-sectional study conducted in the Delta State University Teaching Hospital Oghara, Ethiope west local government area Delta State, Nigeria. A 180-bed capacity hospital with five major wards: they are A/E, internal medicine, surgery, paediatrics, obstetrics and gynaecology. The hospital is equipped with a modern laboratory manned by pathologists, laboratory technologist and technicians.

STUDY POPULATION

Inclusion criteria

Results of urine microscopy/culture and sensitivity tests performed on patients over a 6 month period were obtained from the laboratory register.

Exclusion criteria: However, the results of pregnant women and children under 10years of age were excluded.

Variables
Variables collected included age and sex of patients; presence or not of urinary abnormalities such as haematuria, pyuria, crystals, casts, cultured micro-organism and antibiotic sensitivity profile.

Data source/measurement
All data were retrieved from the hospital microbiology laboratory registers. Urine microscopy is performed in the hospital laboratory using a light microscope and urine cultured on usual culture media.

Bias
Results of tests carried in the laboratory are usually checked and reported by at least laboratory scientist, and counter-signed by a clinical microbiologist. Information was collected using a data sheet and transferred to an electronic spreadsheet; both authors checked data for errors, double entries and missing information.

Study size
Results of all (n=153) urine microscopy culture and sensitivity tests done during the 6 months were collated.

Quantitative variables
Haematuria was regarded as evidence > three red cells/high power field, pyuria as having ≥three white cells/high power field of unspun urine or ≥ ten white cells per cubic millimetre in a urine specimen.[15,16] Urinary tract infection (UTI) was defined as having a cultured organism on media.

Statistical analysis
Data was entered into Excel spread sheet and cross checked by both authors. Data was analyzed using IBM SPSS version *22*. Categorical variables were presented as frequencies and percentages, while continuous variables were presented as mean and standard deviation. The Chi-square or Fisher's exact test was used to determine any association between demographic factors (age, sex) and urinary abnormalities (haematuria, pyuria and UTI). Level of significance was set as <0.05.

RESULTS
A hundred and fifty-three patient results were assessed. The mean age of patients was 49±17 years, and the majority were males (54%).

The frequency of haematuria and pyuria was 19% and 52.3% respectively. Haematuria was frequent in males compared to females (24% vs 14.3%, X^2=5.16, P= 0.023); while 64% of females compared to 42% males had pyuria (X^2=9.09, P= 0.003), see table 1 and 2. Casts were seen in only 2.7%, out of which 1.4% and 1.3% had hyaline and granular casts respectively. Crystals were seen in 6.5%, commonest being calcium oxalate (3.9%).

The prevalence of UTI was 47.1%, with the highest frequencies in the >70yrs age groups (78%). More males (24.7%) compared to females (22.9%) had UTI (X^2=3.35, P= 0.188), see table 3. The organisms isolated were E.coli (40.2%), Klebsiella (19.4%), Candida (9.7%), and Pseudomonas was grown in 5.6%. (Fig 1). Of the 29 cases with E.coli growth, 14 were sensitive to Amoxicillin/Clavulanic acid (Augmentin), and 11 were sensitive to Ceftriaxone (See table 4)

Table 1: Relationship Between Age, Sex and Haematuria

VARIABLES	Haematuria	No Haematuria	Total	P-value
Age (years)	n (%)	n (%)	n (%)	0.438*
10-19	1(20.0)	4 (80.0)	5 (100.0)	
20-29	2 (11.7)	15 (88.3)	17 (100.0)	
30-39	5 (15.6)	32 (84.4)	37 (100.0)	
40-49	4 (25.0)	16 (75.0)	20 (100.0)	
50-59	2 (16.7)	10 (83.3)	12 (100.0)	
60-69	10 (25.6)	29 (74.4)	39 (100.0)	
70-79	5 (27.8)	13 (72.2)	18 (100.0)	
80-89	0 (0.0)	4 (100.0)	4 (100.0)	
90-99	0 (0.0)	1 (100.0)	1 (100.0)	
Total	29 (19.0)	124 (81.0)	153 (100.0)	
SEX				0.023
Male	17 (24.6)	52 (76.4)	69 (100.0)	
Female	12 (14.3)	72 (85.7)	84 (100.0)	
Total	29 (19.0)	124 (81.0)	153 (100.0)	

** Fishers Exact Test*

Table 2: Relationship Between Age, Sex and Pyuria

VARIABLES	Pyuria	No Pyuria	Total	P-value
Age (years)	n (%)	n (%)	n (%)	< 0.001*
10-19	3 (60.0)	2 (40.0)	5 (100.0)	
20-29	5 (29.4)	12 (70.6)	17 (100.0)	
30-39	16 (43.2)	21 (56.8)	37 (100.0)	
40-49	8 (40.0)	12 (60.0)	20 (100.0)	
50-59	12 (100.0)	0 (0.0)	12 (100.0)	
60-69	20 (51.3)	19 (48.7)	39 (100.0)	
70-79	14 (77.8)	4 (22.2)	18 (100.0)	
80-89	1 (25.0)	3 (75.0)	4 (100.0)	
90-99	1 (100.0)	0 (0.0)	1 (100.0)	
Total	80 (52.3)	73 (47.7)	153 (100.0)	
SEX				0.003
Male	33 (41.8)	46 (58.2)	79 (100.0)	
Female	47 (63.5)	27 (36.5)	74 (100.0)	
Total	80 (52.3)	73 (47.7)	153 (100.0)	

** Fishers Exact Test*

Table 3:Relationship Between Age, Sex and UTI

VARIABLES	UTI	No UTI	Total	P-value
Age (years)	**n (%)**	**n (%)**	**n (%)**	0.016*
10-19	2 (40.0)	3 (60.0)	5 (100.0)	
20-29	3 (17.7)	14 (82.4)	17 (100.0)	
30-39	14 (37.8)	23 (62.1)	37 (100.0)	
40-49	12 (60.0)	8 (40.0)	20 (100.0)	
50-59	4 (33.3)	8 (66.7)	12 (100.0)	
60-69	20 (51.3)	19 (48.7)	39 (100.0)	
70-79	14 (77.8)	4 (22.2)	18 (100.0)	
80-89	3 (75.0)	1 (25.0)	4 (100.0)	
90-99	0 (0.0)	1 (100.0)	1 (100.0)	
Total	72 (47.1)	81 (52.9)	153 (100.0)	
SEX				0.188
Male	37 (53.6)	32 (46.4)	69 (100.0)	

Table 4: Sensitivity Pattern of Organisms to Antibiotics

ORGANISM	TOTAL n(%)	AMOX/CLAV n(%)	OFL n(%)	GENT n(%)	IMP n(%)	NITRO n(%)	CAZ n(%)	CRX n(%)
E.COLI	29 (100.0)	14(48.3)	5(17.2)	5(17.2)	3(10.3)	3(10.0)	9(31.0)	11(37.9)
KLEBSIELLA	14 (100.0)	2(14.3)	1(7.1)	0(0.0)	3(21.4)	1(7.1)	0(0.0)	3(21.4)
P. MIRABILIS	7(100.0)	0(0.0)	0(0.0)	0(0.0)	2(100.0)	0(0.0)	0(0.0)	0(0.0)
STAPH. AUREUS	4(100.0)	3(75.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	4(100.0)
ENTEROBACTER	5(100.0)	4(80.0)	0(0.0)	0(0.0)	2(40.0)	0(0.0)	0(0.0)	1(20.0)
AEROMONAS	1(100.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
PSEUDOMONAS	4(100.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
CITROBACTER	4(100.0)	3(75.0)	1(25.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)
CANDIDA	7(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
ACTINOBACTER	2(100.0)	0(0.0)	1(50.0)	1(50.0)	0(0.0)	0(0.0)	1(50.0)	2(100.0)

KEY: AMOX/CLAV – Amoxycillin/Clavulanic acid, OFL – Ofloxacin, GENT – Genticin, IMP – Imipenem, NITRO – Nitrfurantoin, CAZ-Ceftazidime, CRX – Ceftriaxone.

DISCUSSION

This retrospective study of urine microscopic findings in a tertiary hospital located in a low-resource country revealed that the diagnosis of haematuria and pyuria are commoner compared to crystalluria or urinary casts. UTI were found in almost half of the participants' results reviewed, with E.coli being the commonest organism cultured.

The prevalence of haematuria in our study was 19%; Aminu et al. in a hospital-based study in the northern part of Nigeria reported a prevalence of 11% among HIV positive patients, while Braimoh et al. reported a prevalence of 1.7% amongst asymptomatic adults.[17,18] Prevalence of pyuria in our study was 52.3%, and researchers in the eastern part of Nigeria reported a similar prevalence of 52%. [19] Prevalence of casts was 2.7% in this study, hyaline and granular cast were the only casts observed. The prevalence of crystalluria was 3.9% in this study, but Verdesca et al. reported a prevalence of 8.2% using a Fourier transform infrared microspectroscopy.[20] Prevalence of UTI reported in this study (47.1%) is similar to earlier reports from Nigeria. A hospital-based study in southern Nigeria reported a UTI prevalence of 48%, while Bankole et al. reported a prevalence of 39.69% from a community-based study.[21,22] Orrett et al. in Trinidad reported a prevalence of 49%.[23]

Haematuria was commoner in older males compared to females. A possible reason could be that older males are more prone to benign prostatic hypertrophy, UTI, and other urological

abnormalities.[24] Pyuria was more frequent in patients over 50 years than younger patients, possibly because older patients are not as immunocompetent as the young. Furthermore older patients are more likely to be hospitalised and often require an indwelling catheter, which increases the risk for infections. Pyuria was significantly commoner in females compared to males, and Shipman et al. similarly observed this.[25]

This study showed UTI was commoner in adults aged > 70years. A study by Mattina, Deflorio et al. in Italy also reported a higher prevalence in patients age > 60years, [26] however Sekharan et al. studied females in Tanzania and reported a higher prevalence in younger age groups 18-29 years.[27] Surprisingly, UTI was commoner in males in this study, although it did not reach statistical significance. Older males with obstructive uropathy are predisposed to recurrent UTI, males predominated in this study and two-thirds of the patients studied were over the age of 40years. Other authors have reported a higher prevalence in females,[26,28] and the reasons are due to the short urethras in females, the proximity of the urethra opening to the vaginal orifice and hygiene to name a few. Expectedly, the most common organism isolated was the E.coli and was most sensitive to Amoxycillin/Clavulanic acid. Proteus had 100% sensitivity to Imipenem and Staph. Aureus was 100% sensitive to ceftriaxone. The reduced sensitivity of E.coli to Ofloxacin and Genticin may be partly explained by self-medication amongst patients, particularly with less expensive medication before presenting to tertiary hospitals; the use of antibiotics in hospitals before adequate investigations as well as incomplete treatment due to non-adherence are other factors that encourage antibiotic resistance.

The most typical crystal isolated in this study was the calcium oxalate which is in keeping with previous literature,[8,11] Urinary casts and crystals were rarely seen in this study, possibly because of urine examination was performed using a light microscope without phase-contrast enhancement.

This study was retrospective and hospital based, and so findings may not be generalisable to the population. Being a hospital-based study, finding may overestimate the true frequency of urinary abnormalities, particularly UTI, haematuria; however overall, our results are comparable to previously reported estimates and are a meaningful contribution to knowledge in the field.

CONCLUSION

Haematuria and pyuria are more frequent abnormalities compared to casts and crystalluria. Haematuria was significantly commoner in males while more females had pyuria. E.coli was the most common organism implicated with fair sensitivity to amoxicillin/clavulanic acid and cephalosporins. Although microscopic urine examination is an indispensable tool in nephrology care, to benefit from the wealth of information obtainable, it has to be appropriately executed by trained personnel with the right protocols and equipment. There is need for phase-contrast microscopes in Nigerian institutions to improve diagnosis of urine sediments. Future local studies on microscopic urine examination using phase-contrast microscopes are required.

Competing Interest

Authors declare no competing interests.

Authors' Contributions

Roy Aghwana developed the research protocol, collected data, and wrote the first draft of the manuscript.

Oritseweyinmi Edema reviewed the introduction and methodology of the manuscript.
Ogochukwu Okoye conceptualized the research, and critically reviewed and edited the manuscript draft.

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Bacteria Associated with Hospital Waste of Federal University Teaching Hospital, Owerri

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ABSTRACT

Introduction: Hospital waste management is critical for minimizing health risks and environmental damage, particularly in developing countries where inadequate training and resources exacerbate these issues. The Federal University Teaching Hospital, Owerri, serves as a case study to determine the bacteria associated with hospital waste. Objectives: This study aimed to determine the bacterial contamination in hospital waste.

Materials and Methods: An experimental study design was employed. The hospital waste samples were analysed for bacterial contamination via microbiological standards.

Results: The infectious waste had the highest total viable count (9.86±0.28 CFU/g), indicating a high level of microbial presence, whereas the sharps waste presented a much lower total viable count of 5.40±0.52 CFU/g. The highest number of infectious waste isolates was 45 (30.2), followed by general waste 41 (27.5) and chemical waste 8 (5.4). Among the infectious waste, *Enterobacter cloacae* (50%) was the most common bacteria, followed by *Staphylococcus Scuri* ssp. *Lentus* (32%). Among the sharps waste, *Staphylococcus gallinarum* (12%) was the most common, followed by *Staphylococcus xylosus* and *Escherichia coli* (11%). In general, waste, *Pantoea ananatis* (100%) was the most prevalent bacteria, followed by *Escherichia coli* (38.9%), and the least prevalent bacterium was *Staphylococcus Scuri* ssp. *Lentus* (20%).

Conclusion: This study highlights the need to mitigate the spread of resistant pathogens. Continuous monitoring and updated waste management strategies are essential to protect public health and the environment.

Keywords: Hospital waste, bacteria, susceptibility, distribution and susceptibility

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INTRODUCTION

Hospitals are non-replaceable institutions for mankind's health and well-being with universal provision of services that ensure round-the-clock attention to intricate health problems [1]. However, waste management in hospitals has become a real issue due to the generation of obvious health risks and environmental issues [2]. It remains a critical issue, largely in developing countries where many healthcare centres are confronted with technological, economic development and social constraints; and the lack of proper training of healthcare workers responsible for waste disposal [3]. Poor handling and disposal methods embraced in the disposal and handling of medical waste are increasing

health and environmental hazards due to the infectious nature and pungent smell of the waste[4]. Nigeria hospital waste management has not received the attention it should have due to insufficient economic resources, poor policy regimes, inadequate public infrastructure, and insufficient other key capacities [3]. Approximately 15% of waste from hospitals is hazardous; 85% of the waste is non-hazardous [5].

Disease-causing microorganisms in unprocessed medical waste can percolate as well as contaminate soil and surface waters [6]. It was revealed through a WHO/UNICEF joint 2015 survey of 24 sampled countries that fewer than a simple majority (58%) were reporting adequate management of medical waste [7]. Dispose of improperly shredded medical trash with no treatment, resulting in percolation into the ground as well as contaminating soil and aquifers with toxic chemicals or drugs. Failure in burn or incineration releases harmful substances such as furans and dioxins into the environment [8]. Hospital waste management is now an essential issue globally, especially in light of antimicrobial resistance (AMR), which has been recognized as one of the most pressing global public health concerns.

According to the Geneva-based World Health Organization (WHO), antibiotic resistance is increasing to unprecedented levels around the globe and contributing to increased rates of morbidity and mortality [9]. There had already been 929,000 directly AMR-related deaths in the year 2019 alone and an overall related impact due to AMR amounting to an estimated 3.57 million lives. The six major pathogens responsible for these deaths are Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa [10]. According to the World Health Organization, by 2050 it may increase to 10 million. This study was therefore aimed at determining the bacteria associated with hospital waste of Federal University Teaching Hospital, Owerri hospital waste.

MATERIALS AND METHODS

Study Design

The study employed an experimental design. The experimental design was used to study the resistant pathogens associated with the waste at Federal University Teaching Hospital, Owerri.

Area of Study

The Federal University Teaching Hospital is located in Owerri city, Imo State, southeastern Nigeria. In 1903, Federal University Teaching Hospital, Owerri was founded as a colonial dispensary[11]. It was promoted to a district hospital, then a general hospital, before finally becoming a Federal Medical Centre in 1995 [12].

Collection of hospital waste sample

The waste was received from color-coded and labeled containers that indicated the type of waste that they contained. Such waste was traditionally sorted at source into categories including infectious, pathological, sharps, chemicals, pharmaceuticals, and general waste. It was facilitated through staff that had been trained on how to dispose of waste properly. 24 samples were taken in total. 4 samples were taken on average from each based-on categories like infectious waste, pathological waste, sharps, chemical waste, pharmaceutical waste, and general waste. Personal protective gear (PPE; i.e. gloves and laboratory coats) was worn before taking samples to provide a clean environment and safety from danger. The samples were aseptically collected using a sterile collection vessel to avoid further contamination by the environment while collecting. Each sample was labeled with the correct data, which included

name of waste collected, where sampled and collection day.

Microbiological analysis

Enumeration and identification of bacteria were carried out aseptically in the Laboratory of Infectious Disease and Molecular Epidemiology at the Department of Public Health, Federal University of Technology, Owerri, Imo State, Nigeria. The waste samples were initially separated in a Biosafety Cabinet II. One gram of hospital solid waste was placed in a 5 ml sterile Stuart transport medium tube, and the sample was allowed to wet completely. The tube was tightly closed to prevent leakage and contamination. Vortexing was performed on the samples, and 90 µL of the eluate was used to inoculate the culture for each plate/test condition [13]. The samples were inoculated in triplicate in the following culture media using the spread plate method: MacConkey agar with cefotaxime (Hardy), Nutrient agar (Hardy), mannitol salt agar (Hardy), and blood agar (Hardy). The plates were incubated at 37°C for up to 48 hours in an air incubator [14]. Once growth on the culture media was obtained, plates were counted using an electronic colony counter, and all isolates were subcultured and purified and then stored at −80°C in Tryptic Soy Broth containing a glycerol concentration of 20% (v/v). Isolated microbes were identified based on a combination of morphological, biochemical, and microscopic examination of colony morphology, cell shape, and size, which provided initial clues for identification [15].

Antimicrobial susceptibility testing (AST)

The susceptibility of the pathogens isolated was tested against a series of antibiotics. It was carried out using Kirby-Bauer Disk Diffusion Susceptibility Test [16], according to the Clinical and Laboratory Standards Institute guidelines[17]. A fresh subculture of each isolate was prepared on nutrient agar. A sterile straight wire was used to touch 5 colonies of every isolate, and they were then suspended into a sterile Bijou bottle containing 5 mL peptone water (Lab M), which was subsequently incubated overnight at 37°C. Overnight broth cultures were diluted in sterile saline to 10^6 colony-forming units/mL by comparing the inoculum turbidity with 0.5 McFarland turbidity standards. A sterile cotton-tip applicator was brought into contact with a standard inoculum and used to inoculate air-dried plates of Mueller-Hinton agar (Oxoid, England). Individual sterile antibiotic discs were inoculated onto each plate and incubated aerobically for 24 h at 37°C. The antibiotics used were levofloxacin (LEV, 30 µg), cefalexin (30 µg, CN) ciprofloxacin (10 µg, CIP), erythromycin (10 µg, E), ampicillin-sulbactam (30 µg, SAM), sulfamethoxazole (125 µg, SXT), cefotoxine (10 µg, CTX), tetracycline (30 µg, TE), ampicillin (10 µg, AMP) and amikacin (30 µg, AK). The diameter of the zone of inhibition of every isolate to the disc was measured with a calibrated ruler according to the standardized CLSI 2021 guidelines [18].

Method of Data Analysis

The data were analysed via the Statistical Package for Social Sciences (SPSS) computer software version 25. Frequencies and percentages were used to analyse the variables.

RESULTS

Table 1 presents the mean bacterial counts of various types of hospital wastes, i.e., the total viable count, coliform count, and total selective bacterial count, expressed as colony-forming units per gram (CFU/g). Infectious waste contained the greatest total viable count (9.86±0.28 CFU/g) with a significant presence of microbes. It has a coliform count of 3.40±0.31 CFU/g and a total bacterial count of 4.60±0.20 CFU/g, showing high microbial contamination and infection risk. The pharmaceutical waste had

a lower total viable count of 2.40±0.22 CFU/g. It has a coliform count of 1.10±0.20 CFU/g and a total bacterial count of 2.10±0.10 CFU/g, showing relatively low levels of microbial contamination.

Cultural Morphology and Biochemical Characteristics of the Bacterial Isolates

Table 2 provides detailed information on the cultural morphology, microscopic characteristics, biochemical characteristics, and carbohydrate utilization profiles of various bacterial isolates found in the hospital waste. The following bacteria were isolated: *Raoultella ornithinolytica, Staphylococcus gallinarum*, *Enterococcus cloacae, Staphylococcus Scuri* ssp. *Lentus, Escherichia coli*, *Staphylococcus xylosus*, *Klebsiella pneumonia* and *Pantoea ananatis*

Distribution of bacterial isolates among the various types of hospital waste

Table 3 shows the distribution of bacterial isolates among the various types of hospital waste; infectious waste had the highest number of isolates at 45 (30.2), followed by general waste at 41 (27.5) and chemical waste at 8 (5.4). Among the infectious waste, *Enterobacter cloacae* (50%) was the most common bacteria, followed by *Staphylococcus Scuri* ssp. *Lentus* (32%), and the least common species was *Pantoea ananatis*, which was absent. Among the sharps waste, *Staphylococcus gallinarum* (12%) was the most common, followed by *Staphylococcus xylosus* and *Escherichia coli* (11%). In general waste, *Pantoea ananatis* (100%) was the most prevalent bacteria, followed by *Escherichia coli* (38.9%), and the least prevalent bacterium was *Staphylococcus Scuri* ssp. *Lentus* (20%).Among the bacteria subjected to antibiotic susceptibility testing (AST), the highest number of *Staphylococcus gallinarum* (n=25) were found to be sensitive to amikacin (24) but resistant to other antibiotics used. All 30 isolates of *Raoultella ornithinolytica* were resistant to antibiotics, except for amikacin, for which *Raoultella ornithinolytica was* sensitive. The isolates of *Klebsiella pneumoniae* (n=20) were sensitive to the entire antibiotic used. The isolates of *Pantoea ananatis* (n=2) were sensitive to all the antibiotics except for ampicillin, which produced resistance, whereas the *Escherichia coli* isolates (n=18) were resistant to trimethoprim and ampicillin. The bacterial isolates were highly resistant to ampicillin, with the exception of *Klebsiella pneumoniae,* followed by tetracycline and trimethoprim (Table 4).

Table 1: Mean bacterial counts of the various types of hospital waste

Waste Type	**Total viable count** (cfu/g) × 10^2	**Coliform count** (cfu/g) × 10^1	**Selective bacteria count** (cfu/g) × 10^2
Infectious Waste	9.86±0.28	3.40±0.31	4.60±0.20
Sharps	5.40±0.52	1.20±0.22	2.15±0.14
Pathological Waste	6.45±0.18	2.20±0.33	3.31±0.40
Chemical Waste	4.10±0.52	0.0	1.0±0.20
Pharmaceutical Waste	2.40±0.22	1.10±0.20	2.10±0.10
General Waste	7.86±0.28	2.40±0.32	4.50±0.24

Table 2: Cultural morphology and biochemical characteristics of the bacterial isolates from hospital waste

COLONY FEATURES	MICROSCOPY	BIOCHEMICAL REACTIONS													CARBOHYDRATE UTILIZATION						ORGANISM
	Cell Arrangement	spore	Motility	Capsule	Catalase	Oxidase	Coagulase	Indole	Nitrate	MethylRed	V.P	Urease	H_2S	citrate	Glucose	Sucrose	Lactose	maltose	manitol	xylose	
Smooth circular colonies creamy and butyrous and translucent on Nutrient Agar (NA)	gram-positive group of oval cells	-	-	-	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	*Staphylococcus gallinarum*
Small dark red colonies that ferment lactose on MacConkey Agar (MA)	gram-positive cocci in pairs and some in short chains	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	+	+	*Enterococcus cloacae*
Circular smooth colonies with light-yellow pigments on Mannitol Salt Agar (MSA).	gram-positive group of oval cells, some clustered	-	-	-	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	*Staphylococcus Scuri* ssp. *Lentus*
Small pink shiny smooth colonies on MacConkey Agar (MA).	gram-negative short rods in singles	-	+	-	+	-	-	+	-	+	-	-	-	-	+	±	+	+	+	+	*Escherichia coli*
Circular smooth colonies with light-yellow pigments on Mannitol Salt Agar (MSA).	gram-positive group of oval cells, some clustered	-	-	-	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	*Staphylococcus xylosus*
Large mucoid pink colonies on MA	gram-negative short rod in singles	-	-	+	-	-	-	-	+	-	+	+	-	+	+	-	+	-	-	-	*Klebsiella pneumonia*
Slight pink colonies on MA	gram-negative rods in singles	-	+	+	+	-	-	-	+	-	+	+	-	-	+	+	-	-	+	-	*Pantoea ananatis.*
Large mucoid colonies, pink on MacConkey Agar (MA)	Gram-negative short rods in singles	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	*Raoultella ornithinolytica*

KEY: + = Positive, - = Negative, V.P = Voges-Proskauer, NA = Nutrient Agar, MA = MacConkey Agar, BA = Blood Agar.

Table 3: Distribution of bacterial isolates among the various types of hospital waste

Waste → Type Bacteria	Infectious Waste	Sharps	Pathological Waste	Chemical Waste	Pharmaceutical Waste	General Waste
Raoultella ornithinolytica (n=30)	9 (30.0)	3 (10)	6 (20)	1 (3.3)	4 (13.3)	7 (23.3)
Klebsiella pneumonia (n=20)	5 (25.0)	2 (10.0)	3 (15.0)	2 (10.0)	3 (15.0)	5 (25.0)
Staphylococcus gallinarum (n=25)	7 (28.0)	3 (12.0)	3 (12.0)	3 (12.0)	2 (8.0)	7 (28.0)
Enterobacter cloacae (n=12)	6 (50.0)	0 (0.0)	2 (16.7)	0 (0.0)	0 (0.0)	4 (33.3)
Staphylococcus Scuri ssp. *Lentus* (n=25)	8 (32.0)	2 (8.0)	4 (16.0)	1 (4.0)	2 (8.0)	5 (20.0)
Staphylococcus xylosus (n=17)	5 (29.4)	2 (11.8)	3 (17.6)	1 (5.9)	2 (11.8)	4 (23.5)
Escherichia coli (n=18)	5 (27.8)	2 (11.1)	3 (16.7)	0 (0.0)	1 (5.5)	7 (38.9)
Pantoea ananatis (n=2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
Total=149	45 (30.2)	14(9.4)	24 (16.1)	8 (5.4)	14 (9.4)	41 (27.5)

Table 4: Antibiotic susceptibility pattern of the bacteria isolates from the hospital wastes

Bacteria	LEV (30 µg)		CN (30 µg)		CIP (5 µg)		E (30 µg)		SAM (30 µg)		SXT (125 µg)		CTX (30 µg)		TE (30 µg)		AK (30 µg)		AMP (10 µg)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Raoultella ornithinolytica (n=30)	0	30	0	30	0	30	0	30	0	30	0	30	0	30	0	30	20	10	0	30
Klebsiella pneumonia (n=20)	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	19	1
Staphylococcus gallinarum (n=25)	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	24	1	0	25
Enterobacter cloacae (n=12)	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	9	3
Staphylococcus Scuri ssp. *Lentus* (n=25)	20	5	20	5	22	3	0	25	0	25	0	25	24	1	0	25	22	3	0	25
Staphylococcus xylosus (n=17)	17	0	17	0	17	0	17	0	17	0	17	0	17	0	17	0	17	0	0	17
Escherichia coli (n=18)	18	0	18	0	18	0	18	0	18	0	0	18	18	0	18	0	18	0	0	18
Pantoea ananatis (n=2)	2	0	2	0	2	0	2	0	2	0	2	0	2	0	0	2	2	0	0	2

Levofloxacin (LEV), cefalexin (CN) ciprofloxacin (CIP), erythromycin (E), ampicillin-sulbactam (SAM), sulfamethoxazole (SXT), cefotoxine (CTX), tetracycline (TE), ampicillin (AMP) and amikacin (AK). The interpretative standard for S= Sensitive, R=Resistant is the Clinical and Laboratory Standards Institute.

DISCUSSION

The bacteria associated with hospital waste of Federal University Teaching Hospital, Owerri was studied.

The infectious waste demonstrated the greatest extent of microbial contamination. The high microbial loading is to be anticipated owing to the composition of infectious waste, in which it typically contains bodily fluids, tissues, and other miscellaneous materials that might harbor and spread pathogen [19]. The high microbial load indicates the significance of strict waste management practices, such as the use of

incineration and autoclaving, to lower the risk of infection among healthcare professionals and the public. Sharps, being a physical hazard, have much lower microbial contamination levels, which are single-use and discarded immediately upon use, limiting the potential for microbial growth [20], [21]. Nevertheless, the physical hazard posed by sharps necessitates rigorous disposal procedures, as seen in the use of incineration and encapsulation by the hospital. Pathological waste had viable bacteria, coliform, and bacterial, with moderate microbial contamination. This type of waste, body parts and human tissue, can be a source of pathogens if not handled. Autoclaving and incineration of pathological waste in the hospital are optimal practices because these will be efficient in reducing microbial loads and inhibiting infection transmission [22]. Chemical waste had the least microbial contamination, and no detectable coliform or total bacteria were found. The low microbial counts show the effectiveness of chemical disinfection and the inhibitory effect of chemical agents towards microbial growth. This finding agrees with the fact that chemical waste, if disposed of properly, poses a reduced risk of biological contamination [23; 2]. Pharmaceutical waste is relatively low in microbial contamination. The low microbial counts show that pharmaceutical waste, particularly when incinerated, is of low risk towards causing infection. However, improper wastage of medicinal waste can lead to environmental pollution and the development of antimicrobial resistance [24]. The total waste had high to moderate level of microbial contamination. General waste is not hazardous; however, presence of high-level microbial contamination puts into prominence proper segregation and disposal of waste methods to prevent spread of infection both in the community and hospitals [25; 19]. The findings of this study point towards some of the areas of hospital practice of waste improvement. The high microbial load in infectious and general wastes reflect the need for more training in health workers on waste handling and segregation [22].

These have isolated *Staphylococcus gallinarum, Enterococcus cloacae, Staphylococcus Scuri ssp. Lentus, Escherichia coli, Staphylococcus xylosus, Klebsiella pneumonia* and *Pantoea ananatis*. This is consistent with previous research that has identified these bacteria to be prevalent hospital contaminants [26]. The incidence of bacterial isolates in different classes of hospital wastes reflects significant variation in microbial contamination levels, and this is inversely proportional to the type of waste and management. Infectious waste, and the most prominent number of isolates is the most hazardous among them, of which *Enterobacter cloacae* is most prevalent. This finding is concerning, given the reported resistance of Enterobacter cloacae to a number of antibiotics and its association with hospital-acquired infection [27]. The isolation of *Staphylococcus Scuri* ssp from infectious waste also indicates the potential for transmission of resistant organisms, as this species is commonly linked to methicillin and other beta-lactam antibiotic resistance [28]. In sharp waste, *Staphylococcus gallinarum* and *Escherichia coli* were the most common isolates. The relatively lower degree of contamination in sharps waste can be attributed to the sterilization processes that are typically applied for sharp instruments before disposal. Nevertheless, the presence of *Escherichia coli*, a known pathogen with documented cause of urinary tract infection and other healthcare-associated infection, is still a significant risk, particularly where the sharps are not adequately sterilized [29]. General waste, with a mix of materials, had the second-largest number of bacterial isolates. *Pantoea ananatis* was unexpectedly the most prevalent bacterium in general waste, followed by *Escherichia coli* as the second most prevalent. The widespread presence of *Pantoea ananatis* in general waste is especially concerning, given its recent designation as an emerging human pathogen with reported

antibiotic resistance [30]. The occurrence of *Escherichia coli* once again highlights the potential public health risk in the improper disposal and handling of general waste that can serve as a reservoir for resistant pathogens. Chemical waste was least contaminated. This was because of the inherent antimicrobial activity of most chemicals used in hospitals that prevents bacterial growth. However, the presence of any bacterial contamination in chemical waste remains justifiable, as it implies potential weaknesses in segregation or treatment procedures [31].

The antibiotic susceptibility testing (AST) findings point to an issue regarding the prevalence of antibiotic-resistant bacteria in hospital waste. *Staphylococcus gallinarum*, one of the most frequently isolated bacteria, was amikacin-sensitive but resistant to all other antibiotics. This is a concerning trend, as it indicates a narrow spectrum of effective treatment, highlighting the need for strict antibiotic stewardship and infection control practices within the hospital setting. *Raoultella ornithinolytica* was resistant to all but amikacin. This antibiotic profile further burdens treatments and suggests bacteria possess efflux pump mechanisms or antibiotic-modyfying enzymes providing them broad-range resistance [32]. *Klebsiella pneumoniae*, on the other hand, was sensitive to all of the tested antibiotics and therefore, at least among tested isolates, this bacterium is not resistant to these treatments. This finding is opposite to global trends where *Klebsiella pneumoniae* has increasingly become resistant, particularly to carbapenems [33]. *Pantoea ananatis* was ampicillin-resistant but sensitive to other drugs, a trend that can be attributed to the presence of beta-lactamase enzymes that are penicillin-class resistant [34]. *Escherichia coli* isolates are resistant to trimethoprim and ampicillin, as in global increases in resistance among *E. coli* bacteria, particularly in hospital environments where antibiotic pressure is significant [35]. The existence of resistance of these bacteria to antibiotics, particularly ampicillin, is evidence of the prevalent occurrence of resistance genes, most likely as a result of excessive and improper antibiotic usage. The prevailing trend towards resistance, particularly high resistance to ampicillin, tetracycline, and trimethoprim, indicates that these antibiotics are no longer effective in treating infections caused by these pathogens. This resistance can cause more severe healthcare-associated infections and more complicated treatment courses, which require the use of more powerful, often more toxic, or more expensive antibiotics.

CONCLUSION

The study found significant microbial contamination in certain waste types. *Staphylococcus gallinarum* was one of the most frequently isolated bacteria, it was amikacin-sensitive but resistant to all other antibiotics while *Raoultella ornithinolytica* was resistant to all except amikacin. This is a concerning trend, as it indicates a narrow spectrum of effective treatment, highlighting the need for strict antibiotic stewardship and infection control practices within the hospital setting.

Limitations

By focusing exclusively on the Federal University Teaching Hospital in Owerri, the results bear relevance only to this institution and may not be generalized to other hospitals, even within the same locale, where waste management practices and bacterial resistance patterns may significantly differ.

The focus of the study is narrowed to bacteria within the hospital setting, but not the bigger environmental impacts of wastes affecting surrounding communities or ecosystems. More detailed environmental assessments might enhance the findings, but this would require different methods and resources.

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Microanatomy of Term Placenta: Insights from Human and Wistar Rat Studies

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ABSTRACT

Introduction: The placenta is crucial for the development and survival of the maturing foetus. Wistar rat models are commonly used in biomedical research to study human biological responses. However, there is paucity of literature on the histology of Wistar rat placenta in open-access journals.

Materials and Methods: In this study, young adult male and nulliparous female Wistar rats were obtained, acclimatized, and allowed to mate overnight. On the 20th day of gestation, the pregnant rats were euthanized, and their placentas were collected and fixed in 10% neutral buffered formalin. Human placentas were sampled from patients who had normal vaginal deliveries. All samples were processed using haematoxylin-eosin, Masson trichrome, periodic acid-Schiff (PAS), reticulin.

Results and Conclusion: Both human and Wistar rat placentas were haemochorial, meaning the trophoblasts in both species are in direct contact with maternal blood. Differences were observed at the foetal-maternal interface and in the presence of the yolk sac. The highly vascularized labyrinth of the rat placenta features maternal and embryonic blood spaces separated by a trilaminar trophoblast layer and embryonic endothelial cells. In contrast, human-term placental villi consist of thin-walled capillaries lined by endothelial cells, which are surrounded by a syncytiotrophoblast layer and an inconspicuous cytotrophoblastic layer. While the basal plate and chorion of the human and Wistar rat placentas have similar anatomical positions, their morphologies differ. Additionally, the distribution of PAS positivity, collagen, and reticulin fibres varies between the two species. Therefore, caution is advised when extrapolating findings from toxicological studies in rat placentas to humans.

Keywords: Term Placenta, Microanatomy, Human, Wistar rat

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INTRODUCTION

The placenta serves as an interface between the pregnant mother and the developing foetus, playing a vital role in the development and survival of the maturing foetus. Its functions include anchoring the developing foetus to the uterine wall, producing hormones, and facilitating nutrient uptake, oxygen and carbon dioxide exchange, and waste elimination. Placental dysfunction and injury can have adverse effects on the maintenance of pregnancy and foetal development. Given its significance, understanding placental structure and function is crucial for studying developmental diseases and

conditions that impact pregnancy outcomes.[1-8]

The rat placenta consists of both foetal and maternal components. The foetal part includes the yolk sac, chorion, labyrinth zone, and basal zone (also known as the junctional zone). The maternal part includes the decidua and metrial gland. Placental cells originate from the trophectoderm of the embryo and the endometrium of the mother.[7]

The first cell lineages to differentiate in a mammalian embryo are the trophectoderm cells.[2,3,4] The trophectoderm further differentiates so that the portion surrounding the inner cell mass becomes the polar trophoblast, while the part around the blastocoel becomes the mural trophoblast. The polar trophoblast actively proliferates, stimulated by signals from the inner cell mass (ICM). In contrast, mural trophoblast cells are not in close contact with the ICM, and do not benefit from the trophoblast growth factors produced by the embryonic cells. They therefore, exit the mitotic cell cycle, enlarge, and undergo rounds of DNA replication without mitosis, eventually becoming polyploid and forming primary giant cells.[3,4,10,11,12] After implantation, the mural trophectoderm differentiates into primary trophoblastic giant cells, which play a crucial role in regulating decidualization. This is the process by which the maternal endometrium is gradually remodelled into the specialized tissue (decidua) needed to support the conceptus. Furthermore, once the polar trophoblast comes into contact with the endometrium, it begins to proliferate and develop into the extra-embryonic ectoderm and the ectoplacental cone. The edge and centre of the ectoplacental cone then differentiate into secondary trophoblastic giant cells and spongiotrophoblasts, respectively, ultimately forming the basal zone. The chorion, which is the embryonic-side membrane of the ectoplacental cone, fuses with the allantois and eventually differentiates into the labyrinth.[3,6,9,10,11]

Wistar rat models have become a common choice in biomedical research, particularly in Nigeria, due to their physiological and anatomical similarities to humans.[13] A key aspect of understanding rat models for human developmental biology is having a solid grasp of the normal anatomy of the placenta. However, free open-access literature regarding the histology of Wistar rat placenta—both normal and abnormal—is relatively sparse. This study aims to fill this gap by providing a detailed comparative analysis of the foetal part of the term placentae in humans and Wistar rats. Such comparisons will enhance our understanding of placental biology and improve the reliability of Wistar rat models in studying human reproductive health.

METHODS

Young adult male and nulliparous female Wistar rats were obtained and quarantined for one week. They were allowed to mate overnight. The rats were fed pelletized feed and had access to clean water ad libitum. On day 20 of gestation, the pregnant rats were euthanized.[4,9] The placentas were collected, separated, and fixed in 10% neutral buffered formalin. They were then dehydrated using increasing gradients of alcohol, cleared in xylene, and embedded in paraffin wax. This study received approval from the Ethical Review Committee of the University of Ilorin.

Paraffin blocks of human placentas were donated by Dr. Folaranmi from the Department of Anatomic Pathology at the University of Ilorin Teaching Hospital. The placenta samples were obtained from control group patients involved in an earlier study. Participants were recruited from the labour ward, comprising maternal ages ranging from 18 to 40 years, with term deliveries having normal clinical and laboratory parameters.

Participants provided consent for the study, which was also approved by the Ethical Review Committee of the University of Ilorin Teaching Hospital.

Five-micron thick sections were cut using a rotary microtome. The sections were subsequently stained with Haematoxylin and Eosin, Masson's Trichrome (to demonstrate the presence of collagen and fibrin), and Periodic Acid-Schiff (PAS) to highlight polysaccharides, along with a reticulin stain for reticulin fibres.[14,15,16]

Slides were examined using a light microscope, and regions of interest were captured with a camera attached to a light microscope using Amscope 3.7 software. White balance correction was performed using Adobe Photoshop Lightroom (v9.50).

RESULTS

The human and Wistar rat placentae are haemochorial, meaning that trophoblast cells come into direct contact with maternal blood. There are notable differences between rats and humans regarding the histological structure of the placenta which include the foetal-maternal interface and the presence of the yolk sac. The chorionic plate, chorionic villi and basal plate in the human placenta correspond in anatomical position to the chorion, labyrinth, and basal zone in the Wistar rat, respectively.

Haematoxylin-Eosin Staining:

The foetal portion of the Wistar rat placenta comprises the yolk sac, chorion, labyrinth, and basal zone, also known as the junctional zone (Figure 1). The yolk sac has a papillary structure and is attached to the chorion (Figure 1). Its epithelial lining consists of a single layer of cuboidal cells with central, round nuclei, characterized by scalloped edges at the apex. Within the yolk sac are thin-walled, capillary-sized vascular channels (Figure 2).

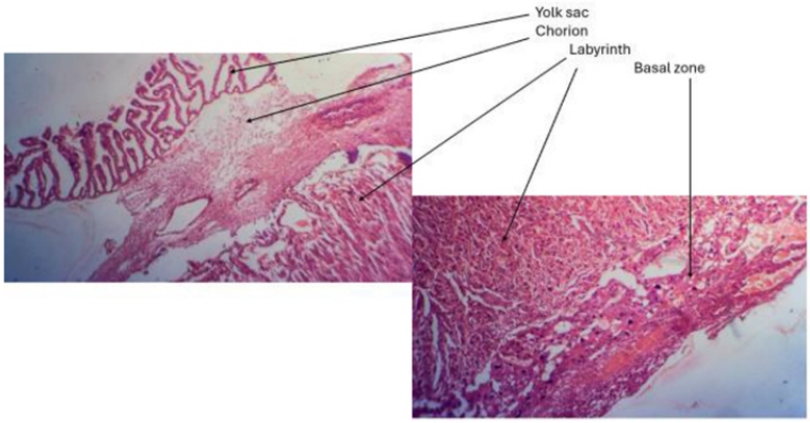

Figure 1. H&E photomicrograph of the layer of the foetal part of Wistar rat placenta. x40

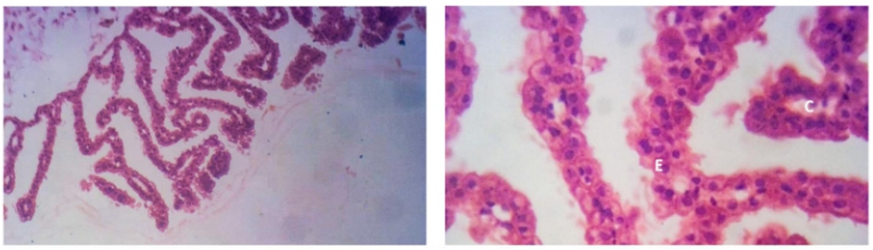

Figure 2. H&E photomicrograph of the yolk sac. Ax100, Bx400.
E: epithelial cell, C: capillary sized vascular channel.

The chorion is made up of plump to spindle-shaped cells with oval to spindle nuclei and abundant eosinophilic cytoplasm (Figure 3A). Its stroma is loose, collagenous and includes thick-walled vascular channels.

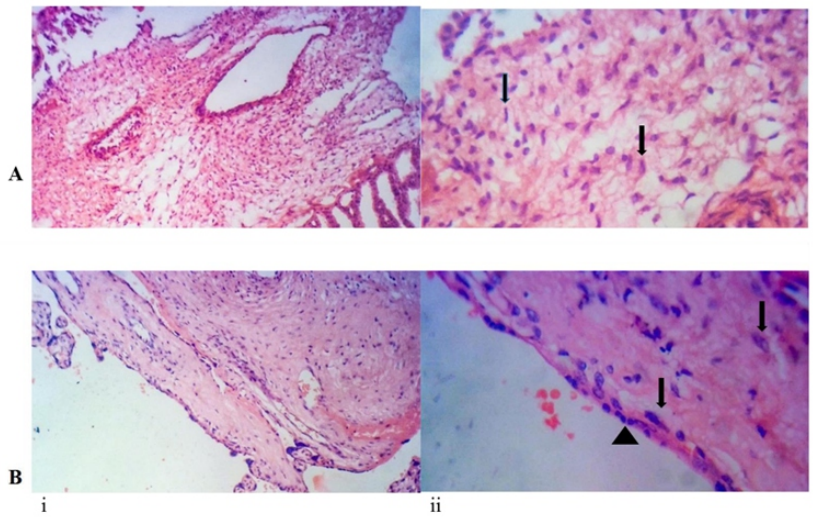

Figure 3.H&E photomicrograph of the A: Chorion in Wistar rat, B: Chorionic plate in human placenta. i: x40, ii: x400. Arrow: Spindle cells; Arrow head: Amnion.

In the highly vascularized labyrinth (Figure 4A), maternal and foetal blood spaces are separated by a trilaminar trophoblast, which includes a single layer of sinusoidal trophoblast lining the maternal sinusoids and two layers of trophoblast cells. The sinusoidal trophoblast cells are relatively large, featuring large basophilic nuclei and ample cytoplasm. The lining of the maternal sinusoids by the trophoblastic cells is not continuous. In contrast, other trophoblastic cells in the trilaminar wall are smaller, with round nuclei, and some are multinucleated. The non-sinusoidal trophoblasts form a continuous layer. Anucleated red blood cells are present within both the maternal sinusoids and foetal capillaries.

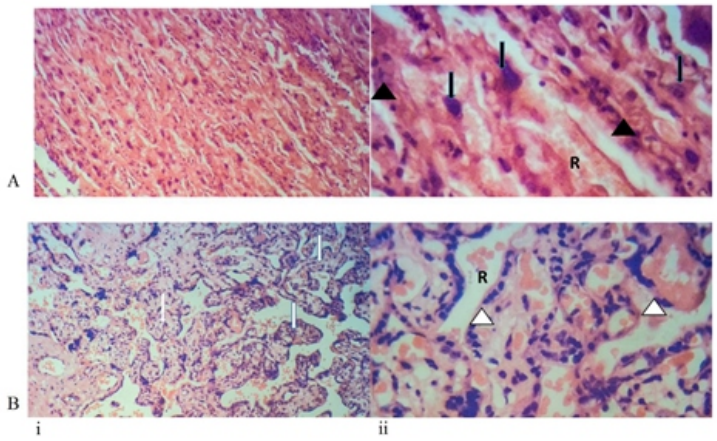

Figure 4. H&E photomicrograph of the A: labyrinth, B: villi. i: x40, ii: x400
Black arrow: sinusoidal trophoblast, black arrow head: trillaminar wall, white arrow: villi, white arrowhead: syncytiotrophoblast. R red blood cells

Beneath the labyrinth lies the basal zone, which contains spongiotrophoblasts and giant trophoblastic cells. Maternal sinuses, lined by giant trophoblastic cells, can also be found within the basal zone (Figure 5A). The giant trophoblasts are large, fairly pleomorphic, and much larger than the sinusoidal trophoblasts, featuring huge basophilic nuclei and abundant eosinophilic cytoplasm. Sheets of spongiotrophoblast cells are found beneath the giant trophoblasts; these spongiotrophoblasts are monomorphic, consisting of plump cells with oval basophilic nuclei and eosinophilic cytoplasm. Numerous capillary-sized blood vessels lined by flattened endothelial cells are observed within the sheets of spongiotrophoblast, along with dilated congested blood vessels.

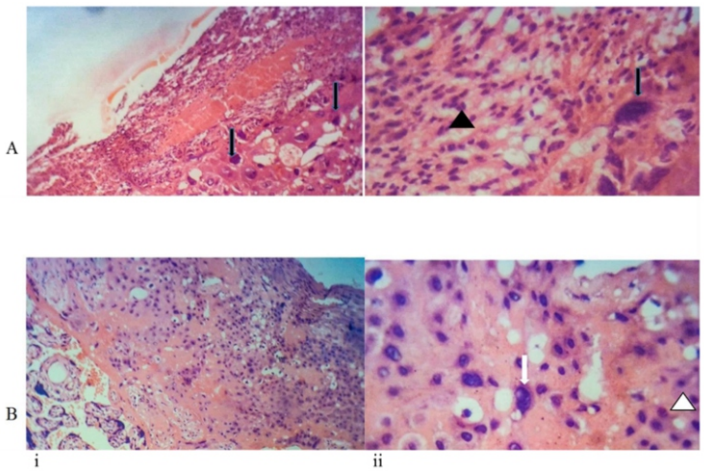

Figure 5. H&E photomicrograph of the A: Basal zone in Wistar rat, B: Basal plate in human. i: x40, ii: x400.
Black arrow: giant trophoblast, black arrow head: spongiotrophoblast, white arrow: extravillious trophoblast, white arrowhead: decidual cell,

The human term placenta consists of the chorionic plate, villous parenchyma, and basal plate (Figure 6). The chorionic plate is formed by the amnion and chorion (Figure 3B). The amnion is a single layer of flattened epithelial cells, while the chorion is fibrocollagenous and contains medium-sized vascular channels.

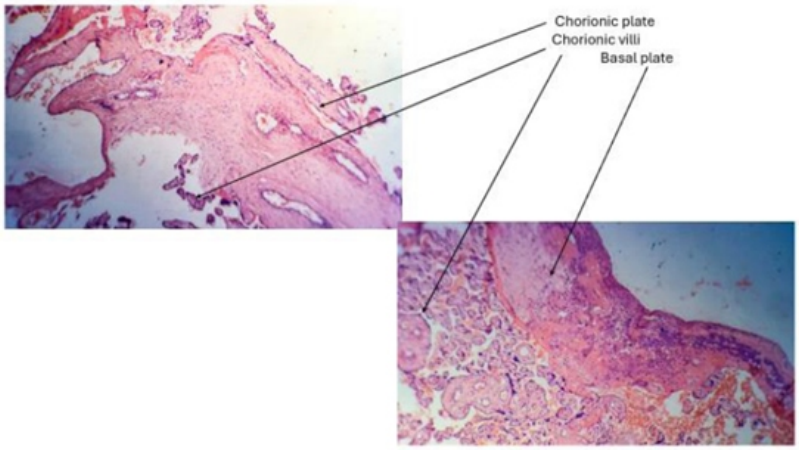

Figure 6. H&E photomicrograph of the human placenta. x40

The chorionic villi, which vary in size and are oval to irregular in shape, are filled with capillaries and lined by a single layer of syncytiotrophoblast (Figure 4B). The villi can be categorized as stem, intermediate, and terminal, with stem and intermediate villi being larger than the smaller terminal villi. The core of the villi is fibrocollagenous and contains spindle cells (fibroblasts) with tapered nuclei and thin-walled capillaries. In terminal villi, there is close opposition between the capillaries in the core and the lining syncytiotrophoblast. Syncytial knots are primarily observed in the terminal villi. Red blood cells are found in the surrounding spaces (maternal sinusoids) adjacent to the villi.

The basal plate (maternal surface) includes extravillous trophoblast cells, decidual cells, fibrin, and vascular channels (Figure 5B). Extravillous trophoblast cells are large, featuring oval basophilic nuclei and scant cytoplasm. In contrast, decidual cells are much smaller, characterized by central oval nuclei and abundant eosinophilic cytoplasm. The fibrin appears as light eosinophilic deposits (Figure 5B).

Masson Trichrome (MT) Staining:

Masson trichrome staining highlights collagen fibres, which can be used to assess and stage fibrosis. In this staining method, collagen appears blue, fresh fibrin stains orange-yellow, mature fibrin is red, and old fibrin is blue. The human chorion is densely collagenized when compared to the chorion in Wistar rats. The labyrinth zone contains very few collagen fibres. The cores of terminal villi show little to no collagen, in contrast to the intermediate and stem villi. In the basal plate of the human placenta, old fibrin appears blue with the Masson trichrome stain, while mature fibrin appears red. In the basal zone of Wistar rat placentas, there is an absence of collagen fibres and fibrin deposition (Figure 7)

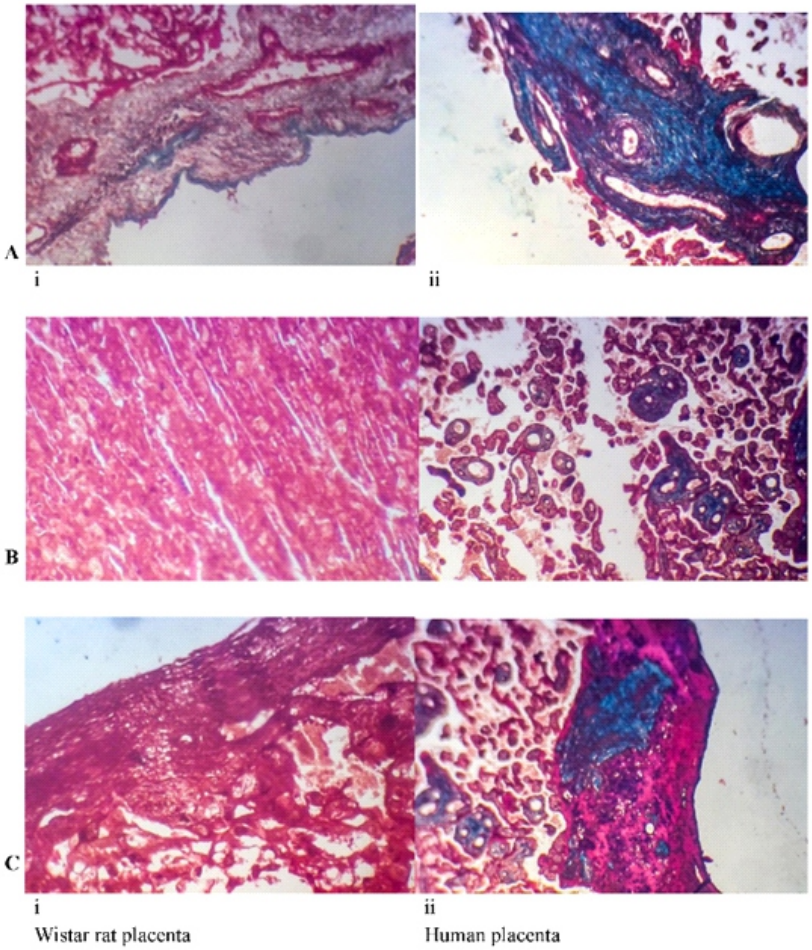

Figure 7. Masson Trichome

A: i. Chorion, ii. Chorionic plate.
B: i. Labyrinth, ii. Villi.
C: i. Basal zone, ii. Basal plate.

Reticulin Staining:

Reticulin staining stains reticular fibres black. Reticular fibres are thin type III collagen fibres. The distribution of reticulin fibres closely mirrors that of collagen fibres. The human chorion demonstrated a dense distribution of reticulin fibres compared to the chorion in Wistar rats. The labyrinth zone has no strands of reticulin fibres located within the trophoblastic septae. Villi exhibit varying amounts of reticulin, with the most abundance found in the stem and intermediate villi. There are no reticulin fibres present in the basal zone of Wistar rat placentas, while the basal plate of the human placenta shows perivascular reticulin fibres (Figure 8).

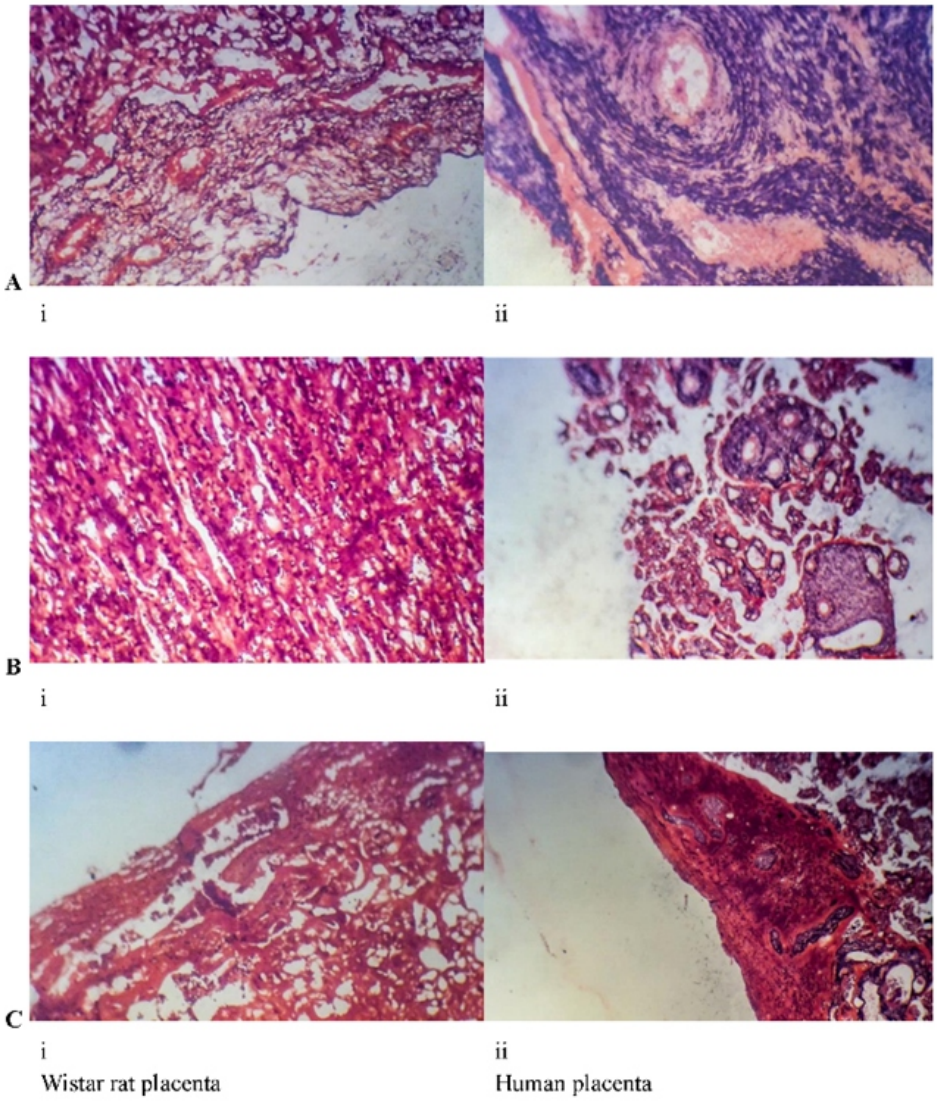

Figure 8. Reticulin stain A: i. Chorion, ii. Chorionic plate.
B: i. Labyrinth, ii. Villi.
C: i. Basal zone, ii. Basal plate.

Periodic Acid-Schiff (PAS) Staining:
PAS stains basement membrane matrix, glycogen, and mucopolysaccharides magenta. The chorion in Wistar rat and chorionic plate demonstrated loose and dense PAS positive areas respectively (Figure 9). The foetal capillary endothelial basement membrane are the only areas showing positive PAS stain in the labyrinth (Figure 9, Figure 10). The cores of stem, intermediate, and terminal villi demonstrated positive reactions to PAS staining. Foci of intracellular and extracellular positive PAS were

seen in the basal zone of Wistar rat. The extracellular PAS positivity were noticed around the vascular channels (Figure 10). Extensive regions of positive PAS staining were found in the basal plate of the human placenta (Figure 9).

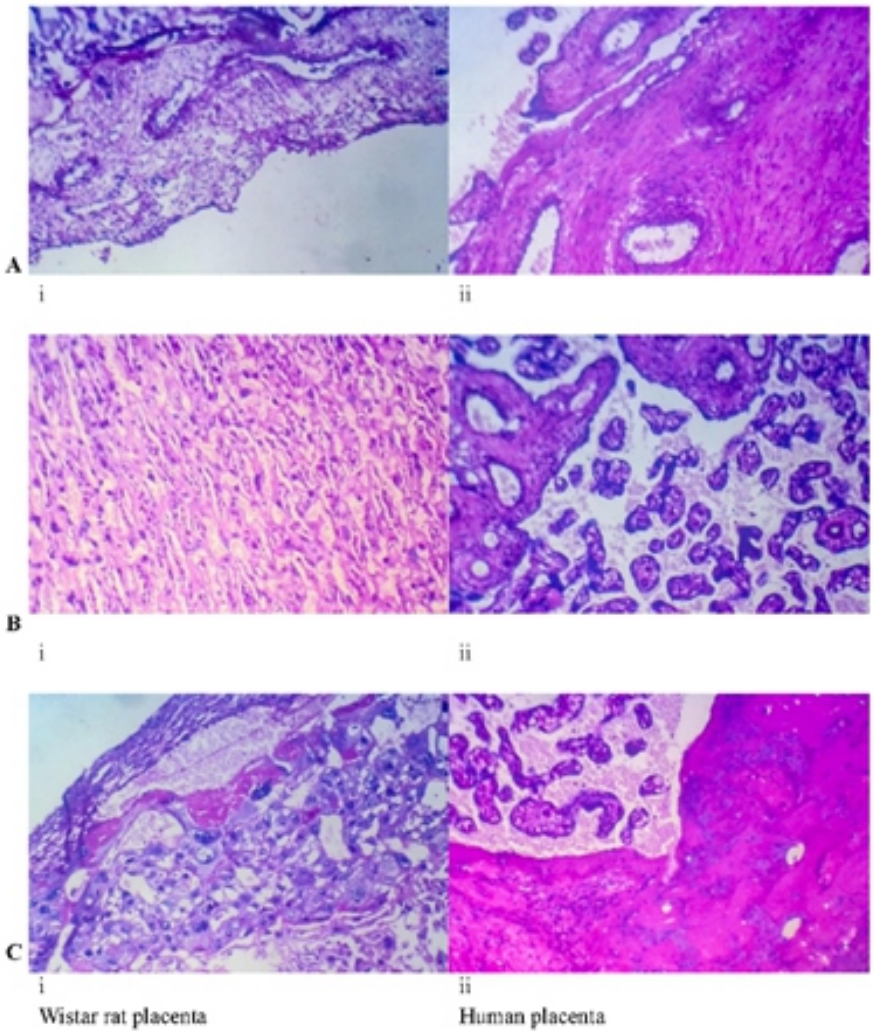

Figure 9. PAS. A: i. Chorion, ii. Chorionic plate.
B: i. Labyrinth, ii. Villi.
C: i. Basal zone, ii. Basal plate.

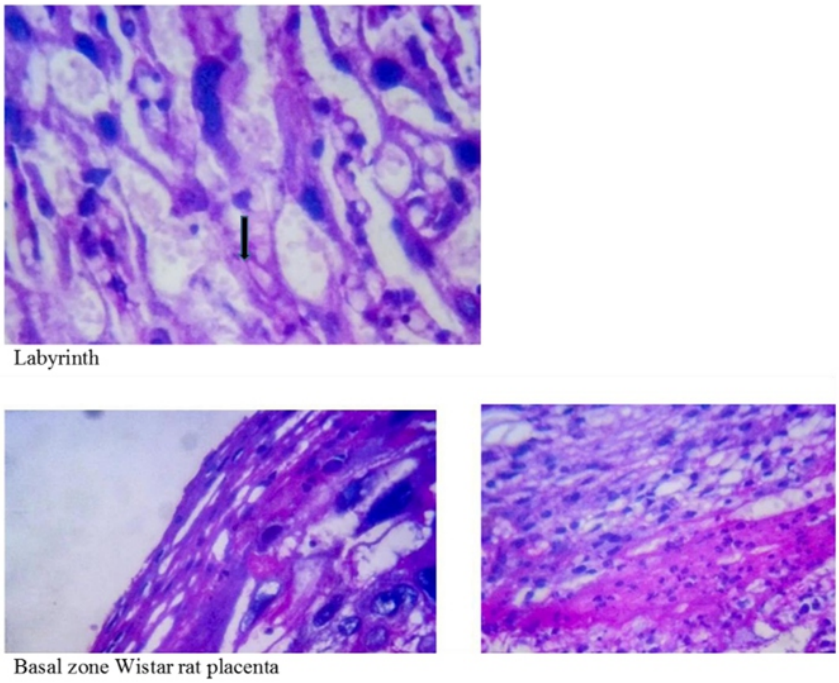

Figure 10. PAS x400.
Black arrow PAS positive endothelial basement membrane.

DISCUSSION

This overview of microscopic placental anatomy serves as a valuable reference for researchers identifying and describing structural changes when working with Wistar rat models of placenta toxicity or diseases.

In mammals, two types of placentas are present during gestation: the yolk sac placenta (or choriovitelline placenta) and the chorioallantoic placenta.[1,4,6,7] The yolk sac placenta serves as a temporary structure during the early stages of pregnancy before the chorioallantoic circulation is established. The yolk sac actively absorbs nutrients from the chorion and the chorionic cavity, transporting them to the embryo through the yolk sac circulation. In humans, as well as in most mammals, the yolk sac placenta becomes vestigial after the first trimester. It remains as a small stalk, floats within the exocoelomic cavity and never directly contacts the chorionic plate. In contrast, in rodents, the yolk sac placenta persists and continues to provide nutrients to the foetus until term. The chorioallantoic placenta is the primary placenta in mammals during the middle to late stages of gestation. It is formed from the endometrium of the mother and the trophectoderm of the embryo.[1,4,6,7]

The chorionic plate serves as the attachment site for the umbilical cord vessels [17]. The umbilical vessels enter and exit the placenta at the chorion. Microscopically, the chorionic plate is composed of dense, collagenized connective tissue that surrounds thick-walled arteries and veins. The human chorionic plate exhibits a high degree of collagenisation, as demonstrated by Masson's trichrome (MT) staining. In contrast, the chorion

of the Wistar rat shows minimal collagen compared to that of humans. The distribution of reticulin fibres is similar to that of the collagen fibres.

Both Wistar rat and human placentas depend on maternal blood from the uterus, which enters the placenta through the decidua and then filters through the labyrinth (in rats) or chorionic villi (in humans).[17,18,19] The labyrinth and villi are responsible for the exchange of oxygen, carbon dioxide, metabolites, and hormones between the foetus and the mother. These structures receive dual blood supply from both the foetal and maternal circulations. Throughout the labyrinth and villi, maternal sinusoids are situated close to foetal vessels. Despite their proximity, the embryonic and maternal circulations never mix at any point during gestation. Although both the labyrinth and villi serve the same function, they differ structurally.[1,4,5,17,18]

The labyrinth contains maternal sinusoids and trophoblastic septa, which are composed of a trilaminar trophoblastic epithelium and foetal capillaries. As pregnancy progresses, there is a decrease in the cellular density within the trophoblastic septa and an increase in the size of the sinusoidal trophoblasts.[4,8,9,20]. The labyrinth lacks collagen fibres, but reticulin staining reveals a few reticulin fibres in the trilaminar septa. Additionally, there is a scarcity of extracellular matrix in the trilaminar septa, as shown by periodic acid-Schiff (PAS) staining.

Villous trees consist of approximately 40 major primary stem villi, which protrude perpendicularly downward from the chorionic plate. Each villus contains vascular channels and fibrocollagenous stroma. The primary villi divide into 4 to 8 secondary stem villi (also known as intermediate villi) that run parallel to the chorionic plate. These secondary villi further subdivide into tertiary stem villi (terminal villi), which form the placental lobules and attach to the basal plate. The terminal villi represent the main area for nutrient and gas exchange in the placenta.[17,18,19,20] They are made up of capillaries and perivascular fibroblasts but have few associated collagen and extracellular matrix as demonstrated with MT and PAS. In contrast, the stem and intermediate villi contain a collagenized core, as demonstrated by MT staining, and do not function as exchange areas within the placenta. PAS stain demonstrated decrease in extracellular matrix from stem to intermediate villi to the terminal villi. Surrounding these villi is a trophoblast bilayer that consists of an outer syncytiotrophoblast layer and an inner cytotrophoblast layer. By midterm to term, the cytotrophoblast becomes less noticeable.

The basal plate is the maternal surface of the placenta. In humans, the basal plate consists of fibrin, extravillous trophoblast cells, decidua basalis, and maternal vascular channels. Endometrial spiral arteries undergo physiological transformation characterized by lumen dilation, invasion of the endothelial lining by extravillous trophoblasts, and the fibrinoid replacement of the muscular and elastic tissue in the arterial wall. As a result, maternal spiral arteries change from high resistance, low capacitance vessels to low resistance, high capacitance vessels.[5,20,21] Fibrinoid deposition was demonstrated by blue and red MT stains in the basal plate of the human placenta. In contrast, no fibrinoid changes were observed in the basal zone of the Wistar rat placenta. The basal zone in Wistar rat placenta is composed of spongiotrophoblasts, glycogen cells, and trophoblastic giant cells.[4,6,7] Glycogen cells, which accumulate glycogen-rich granules, form small clusters and develop into glycogen cell islands, comprising a significant portion of the basal zone on gestational days (GD) 15 and 16. They exhibit nuclear pyknosis after GD 17, leading to basal

zone regression, and by GD 21, almost all glycogen cells disappear while spongiotrophoblasts and trophoblastic giant cells become the main structural components of the basal zone.[4,5] The periodic acid-Schiff (PAS) stain demonstrated few remaining glycogen cells in the basal zone. There is an abundance of extracellular matrix in the basal plate of human placenta as shown by large areas of magenta colour of PAS stain.

In conclusion, this study highlight both the similarities and differences in placental structure between humans and Wistar rats. The haemochorial nature of the placenta in both species indicates functional similarities; however, the significant morphological differences highlight the limitations of directly applying findings from rat toxicology studies to humans.

Conflicts of Interest: The authors declare that they have no competing interests.

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Investigation of Antibacterial Potential of *Bacillus* and *Actinomycetes* Isolated from Soil against Drug-resistant Clinical Isolates

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ABSTRACT

Background: Bacillus and Actinomycetes are well known antibiotic-producing soil isolates. However, there is paucity of information against drug-resistant clinical isolates from Nigeria. This research was therefore designed to investigate the anti-bacterial potential of *Bacillus* and *Actinomycete*s isolated from soil against drug-resistant clinical isolates.

Methods: Soil samples were collected from the *rhizosphere* of Mango trees within Caleb University, Lagos, between January 2024 and March 2024. Targeted isolation of Bacillus and Actinomycetes from the soil samples was done. Clinical isolates of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhi* were identified and subjected to antibiotic susceptibility testing. The crowded plate method was used to screen the antibiotic-producing soil isolates. Ultra-violet (UV) induced mutation was performed on the soil isolates to determine change in antibacterial activity at two different time exposures (2 hours and 4 hours).

Result: The distribution of the soil isolates showed *Bacillus* species (71.40 %) as the most prevalent isolates. Multi-drug resistance was observed among the clinical isolates. Prior to UV exposure, none of the Bacillus isolates exhibited antibiotic- producing activity against the tested clinical isolates unlike Actinomycetes with inhibitory activity against clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli.* However, after UV exposure at 2 hours and 4 hours, all soil isolates demonstrated inhibitory activity against at least one tested clinical isolate.

Conclusion: The inhibitory activity of antibiotic-producing soil bacteria against drug-resistant pathogenic clinical isolates can be enhanced using UV induction.

Keywords: Actinomycetes, antibacterial activity, clinical isolates, multidrug resistant, UV induction

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INTRODUCTION

Among the microorganisms habitats, soils represent a large reservoir and vital global health for the immense diversity of living organisms on earth[1,2], which serves as medium where plant and microbial communities exchange substances[3, 4]. One of the predominant organisms mostly found in the soil is the bacteria, which play vital roles in decomposing, processing, exchange of nutrients, and soil fertility regulation[5, 6, 7]. Findings revealed

that about 30% of the available antibiotics are screened and cultured from the soil, and more than 80% antibiotics used clinically for the treatment of human are isolated from the soil[8]. Antimicrobial resistance (AMR) is one of the greatest world challenges, when antibiotics proven to be effective; has lost their potency due to microorganisms shift over a period[9]. The uncontrolled spread of AMR is a natural process which has evolved from abuse/overuse of antibiotics, and this has constituted to public health threat today[10]. Over time, AMR develops naturally as a result of genetic mutations that could pass from one generation to another. Bacteria develop different mechanisms of resistance which include: antigenic variation, target defense, and suppression of cellular antibiotics[9, 11, 12]. Findings revealed that not all resistant bacteria are capable of producing diseases but achieve pathogenesis through dissemination of gene encoding AMR to new bacteria in a favourable environment[12]. The common means of spreading AMR is the transfer of antibiotic resistant genes. Plasmids, transposons, or integrons and horizontal gene transfer are agents that enhance the transfer of resistant gene(s)[13]. Several natural sources have emerged since the pursuit of novel antibiotics[14]. Most clinically relevant secondary metabolites are best produced by *thermophilic* bacteria; are a biodiverse reservoirs of bioactive compounds. Antibiotics, heat-stable enzymes and cancer agents are valuable compounds of considerable global interest[15, 16]. Thermostable enzymes such as *lipases, amylases, cellulases* are mostly produced in large amount by *thermophilic* bacteria with considerable industrial uses[17].

The genus *Bacillus* are soil inhabitant bacteria, and producers of important antibiotics[18]. *Bacillus* species have a wide spectrum of antimicrobial substances such as *ribosomally* and non-ribosomally synthesized cyclic lipopepeties, bacteriocins and other peptide based compounds[19,20,21]. *Actinomycetes* are notable sources of bioactive compounds which are distributed naturally, and or man-made that play vital roles in organic degradation[22].

Actinomycetes has long history in the production of secondary metabolites, and sources of promising new antibiotics[23, 24, 25]. This research work focused on investigating antibacterial potential in *Bacillus* and *Actinomycetes* isolated from soil against drug-resistant clinical pathogens.

MATERIALS AND METHODS

Study Area

Caleb University main campus is located in Imota, Lagos, Nigeria. Imota's latitude and longitude coordinates are around 6.5833°N and 3.3500°E. The average annual rainfall in Imota is between 1,500 and 2,000 millimetres, with the rainy season typically lasting from March until October. The dry season last from November to February. Imota's average annual temperature ranges from 25°C to 30°C. Imota covers a portion of the Lagos metropolis, which comprises residential areas, commercial spaces, agricultural land, natural vegetation and educational institutions such as Caleb University.

Sample Collection

The soil samples were meticulously collected from *rhizosphere* of Mango trees within Caleb University, Lagos. Sampling was conducted at a depth of at least 10 cm using a calibrator and a sterile spatula. Each sample was carefully stored in a sterile transport box and promptly transferred to the laboratory at room temperature.

Isolation of soil Bacteria

The soil samples underwent air drying by exposing them to 50°C and 70°C for 1 hour in an

oven to target *Bacillus* and *Actinomycetes* respectively. Isolation of *Bacillus* species and *Actinomycetes* was carried out through a serial dilution technique, about 1 g of the soil samples were collected in a sterile container as described by Tabbene et al[18] and Singh et al[23]. A 5-folds serial dilution were performed, samples were diluted progressively: An average of 1 mL from the first tube was mixed with a total 9 mL of distilled water in the next tube, and this process continued until 5th tube. From 5th, an average of 0.1 mL was transferred onto a nutrient agar plate using the spread plate method. Streaking methods were employed for plating on nutrient broth and agar, followed by incubation at 37°C for 24 hours.

Collection of clinical isolates

Clinical isolates of *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* were collected from urine, high vaginal swab, wound and stool samples respectively.

Identification of Isolates

Morphological and biochemical analyses was done to identify soil isolates (*Bacillus* and Actinomycetes) and the collected clinical isolates (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Salmonella typhi*). Morphological analyses was done using Gram-staining and cultural methods. Using the cultural method, growth media: Eosin Methylene Blue Agar (EMB) was used for the isolation of *Escherichia coli* which was isolated from urine sample, and high vaginal swab. Salmonella-Shigella Agar (SSA) was used for the isolation of *Salmonella typhi* isolated from stool sample. Mannitol Salt Agar (MSA) was used for the growth of *Staphylococcus aureus* isolated from stool, and Nutrient Agar was used for the isolation of *Pseudomonas aeruginosa* obtained from wound sample. Biochemical tests, including the citrate utilization test, catalase test, coagulase test, starch hydrolysis test, and urease test.

Antibiotic susceptibility testing of clinical isolates

Isolates of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Salmonella typhi* were subjected to antibiotic susceptibility using fourteen (14) antibiotics. The antibiotics included: pefloxacin (10 µg), gentamicin (10 µg), amoxicillin (30 µg), cefuroxime (25 µg), ceftriaxone (25 µg), ciprofloxacin (20 µg), azithromycin (12 µg), levofloxacin (20 µg), erythromycin (10 µg), co-trimoxazole (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), amoxicillin/clavulanic acid (30 µg), and Ofloxacin (10 µg). The agar diffusion method as described by the Clinical Laboratory Standard Institute[26] was employed.

Primary Screening of Antibiotic-Producing Isolates using Crowded Plate Technique

The crowded plate method was used for the preliminary screening of antibiotic-producing isolates. A total of 0.1 suspension from dilutions 10^{-3} and 10^{-7} was aseptically spread on agar medium with a sterile swab stick. Each clinical test isolates were exposed on the same agar medium, which were then incubated at 37°C for 24 - 48 hours. Following incubation, a clear zone of inhibition found surrounding the test isolate and the soil isolate's colony responsible for the clear zone was then further isolated [27]. Two incubation periods: 24 hours and 48 hours.

Mutation Induction on Antibiotic-Producing Isolates (API)

UV-induced mutation was employed on the API (*Bacillus* and *Actinomycetes*). The isolates were subjected to a UV radiation for 2 hours and 4 hours respectively. Antibiotic production activity was repeated to determine change in activity.

Statistical analysis

The IBM Statistical Package for Social Science (SPSS) version 20 was used to analyse data from the study. The result of antibiotic susceptibility was presented as mean ± standard deviation in bar chart. Results were also presented in percentage on a pie chart.

Ethical Considerations

Ethical approval was obtained from Caleb University Research Ethics Committee with approval number CUL 24/025 according to the declaration of Helsinki.

RESULTS

Isolation and Distribution of Bacterial soil Isolates

The distribution of the soil isolates as presented in Figure 1 showed *Bacillus* species (71.40 %) as the most prevalent isolates. Out of seven (7) bacteria isolates, five (5) of these isolates were identified as *Bacillus* species, and two (2) as *Actinomycetes* species as shown in Plate 1 and 2 respectively.

Antibiotic Susceptibility of Clinical Test Isolates

A panel of conventional antibiotics was used to test the antibiotic susceptibility of four clinical test isolates to determine multi-drug resistance, which included: *Staphylococcus aureus,* S*almonella typhi, Pseudomonas aeruginosa,* and *Escherichia coli* (Figure 2). The results showed that *E. coli* was susceptible to antibiotic; levofloxacin (LEV) with zone diameter of 14 mm. However, it exhibited resistance to antibiotics like ciprofloxacin (CPX), amoxicillin (AM), and amoxicillin/clavulanic acid (AU), with no observable zones of inhibition. *Pseudomonas aeruginosa* displayed resistance to all antibiotics such as ampicillin-clavulanic (AMP-CLAV) and ceftazidime (CEP). *Staphylococcus aureus* was susceptible to antibiotics like azithromycin (AZ), levofloxacin (LEV), erythromycin (E), pefloxacin (PEF), gentamicin (CN), ceftriaxone (C), with zone diameters ranging from 6 mm to 20 mm, but exhibited resistance to antibiotics like cefuroxime (Z), vancomycin (VAN). *Salmonella typhi* demonstrated susceptibility to antibiotic levofloxacin (LEV), with zone diameter of 17 mm, but showed resistance to antibiotics like ciprofloxacin (CPX), amoxicillin (AM), and amoxicillin/clavulanic acid (AU).

Antibiotic-Producing soil isolates (API) using UV-induced mutation

The primary screening of antibiotic-producing isolates (API) was conducted before and after UV radiation exposure using the crowded plate method (Table 1). Prior to UV exposure, none of the *Bacillus* isolates exhibited antibacterial activity against the tested clinical isolates. Nevertheless, both of the *Actinomycetes* isolates were inhibitory to *Pseudomonas aeruginosa* and *Escherichia coli*. However, after UV exposure at different time intervals of 2 hours and 4 hours at two different incubation time of 24 hours and 48 hours; all soil isolates demonstrated inhibitory activity against at least one of the tested clinical isolates (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*, and *Salmonella typhi*). The results interpreted that UV radiation induced mutations in the isolates, leading to the production of antibiotics with inhibitory activity against the tested clinical isolates. The two (2) Actinomycetes isolates: Actino 1 and Actino 2 showed notable antibacterial activity against the tested clinical isolates before and after UV exposure, as shown in Table 1. Actino 1 showed inhibitory activity against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* after both 2 hours and 4 hours UV exposure with 24 hours and 48 hours incubation time (Plate 4.3). Actino 2 also showed inhibitory activity against *Pseudomonas aeruginosa, E. coli* and *Salmonella*

typhi after 2 hours UV exposure and only against *Staphylococcus aureus* at 4 hours UV exposure.

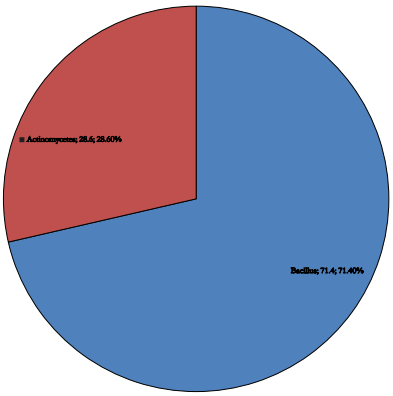

Figure 1: Percentage distribution of soil isolates among *Bacillus* and Actinomycetes

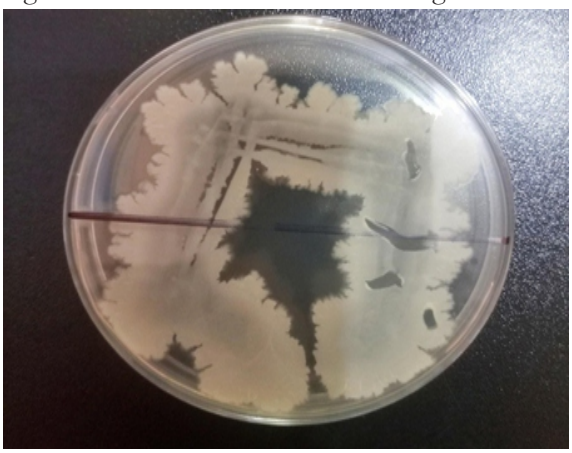

Plate 1: Colony morphology of Actinomycetes

Plate 2**:** Colony morphology of *Bacillus* Sp.

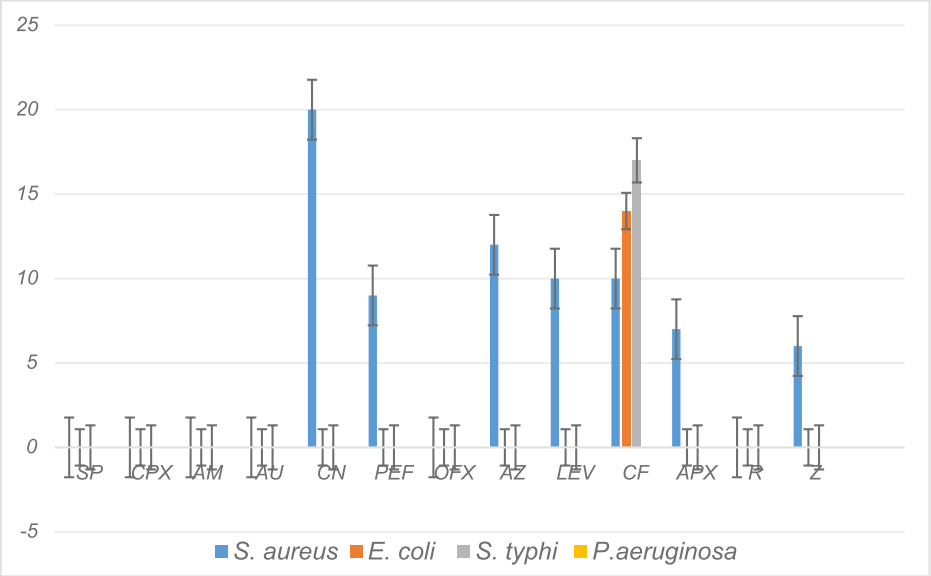

Figure 2: Antibiotic susceptibility of clinical test isolates

Table 1: Primary Screening of Antibiotic-producing Isolates (API) before and after UV exposure

Isolates	Before UV exposure	After 2 hours UV exposure Incubation		After 4 hours UV exposure Incubation		Interpretation
		24hrs	48hrs	24hrs	48hrs	
Bacillus **1**	No activity	Inhibitory to *pseudo, E. coli, Staph*	Inhibitory to *pseudo, E. coli, Staph*	Inhibitory to *pseudo, E. coli, Staph, Sal*	Inhibitory to *pseudo, E. coli, Staph, Sal*	Sensitive to UV
Bacillus **2**	No activity	Inhibitory to *pseudo, E. coli, Staph*	Inhibitory to *E. coli, Staph*	Inhibitory to *pseudo, E. coli, Staph, Sal*	Inhibitory to *pseudo, E. coli, Staph, Sal*	Sensitive to UV
Bacillus **3**	No activity	Inhibitory to *pseudo, E. coli, Staph, Sal*	Inhibitory to *pseudo, E. coli, Staph*	Inhibitory to *Sal, Staph*	Inhibitory to *Sal, Staph*	Sensitive to UV
Bacillus **4**	No activity	Inhibitory to *pseudo, E. coli, Staph, Sal*	Inhibitory to *pseudo, E. coli, Staph, Sal*	Inhibitory to *pseudo*	Inhibitory to *pseudo*	Sensitive to UV
Bacillus **5**	No activity	Inhibitory to *pseudo, E. coli*	Inhibitory to *pseudo, E. coli*	Inhibitory to *pseudo, Sal*	Inhibitory to *Sal*	Sensitive to UV
Actino 1	Inhibitory to *Pseudo, E. coli*	Inhibitory to *Pseudo, E. coli, Staph, Sal*	Inhibitory to *Pseudo, E. coli, Staph, Sal*	Inhibitor y to *Pseudo, E. coli, Staph, Sal*	Inhibitory to *Pseudo, scoli, Staph, Sal*	Sensitive to UV
Actino 2	Inhibitory to *Pseudo, E. coli*	Inhibitory to *Pseudo, E. coli, Sal*	No activity	Inhibitory to *Staph*	No activity	Sensitive to UV

Key: *Pseudo – Pseudomonas aeruginosa E. coli – Escherichia coli Sal – Salmonella typhi Staph – Staphylococcus aureus*

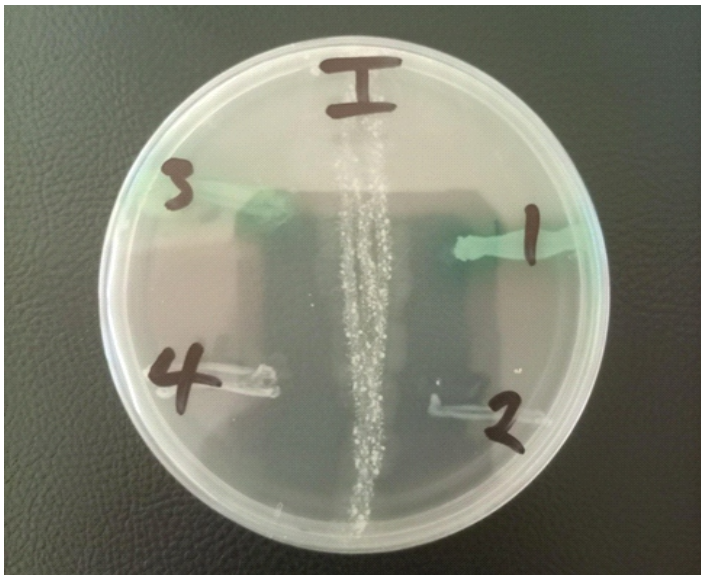

Plate 3: Actinomycetes Crowded plate method after 4 hours of UV exposure.

DISCUSSION

The results, from the study on the variety and distribution of bacteria in soil samples from Caleb University were consistent with Ismail and Ahmed[28]. Higher occurrence of *Bacillus* species compared to Actinomycetes in similar environmental conditions [29]. The prevalence of *Bacillus* species over *Actinomycetes* suggests the resilience and adaptability of *Bacillus* in environments possibly due to their ability to form spores. Comparing these findings with a study conducted, showed the similarities in colony characteristics between *Bacillus* and *Actinomycetes* isolates, such as creamy colonies and distinct enzyme profiles[30]. The smooth white colonies of *Bacillus* spp. contrast with the filamentous appearance of *Actinomycetes* showing their growth patterns. Further biochemical tests revealed differences with *Actinomycetes* having Gram rods and distinct enzyme profiles compared to *Bacillus* isolates. The discovery of multidrug resistance among clinical isolates echoes trends seen in studies[31]. Clinically relevant bacteria like *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa* showed resistance to antibiotics used in this study. This evidence emphasizes the issue of antibiotic resistance and the critical need, for innovative treatment approaches [32,33].

UV-induced mutagenesis is a technique used to generate mutations in bacteria, potentially leading to enhanced antibiotic production. The discovery of UV triggered creation, in this research aligns, with the results discussed by Ibnouf[34] emphasizing how microbial communities adapt to challenges by altering their metabolic pathways and producing different bioactive compounds. A detailed comparison also sheds light on how UV radiation can boost the production of metabolites and but also highlights the potential of these metabolites to improve the combat capabilities of microorganisms against clinically relevant pathogens. In comparison to studies by Chaudhary et al[35], the *Actinomycetes* isolates exhibited comparable or superior antibacterial activity against clinically relevant pathogens both

before and after UV exposure. This reinforces the notion of Actinomycetes as prolific producers of bioactive compounds with therapeutic potential, warranting further exploration for future antimicrobials. The enhanced antibiotic activity observed after UV exposure suggests that these stress conditions may induce the production of novel or more potent antimicrobial compounds.

CONCLUSION

This study showed the efficacy of antibiotic-producing *Bacillus* and *Actinomycetes* against multi-drug resistance clinical isolates. By integrating these findings with existing literature, this study contributes to a comprehensive understanding of antibiotic-producing soil bacteria, antibiotic resistance dynamics, and the biotechnological implications of UV-induced antibiotic production. Future research endeavours may benefit from secondary metabolite production in natural environments such as soil.

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Effect of Methanol Extract of Costus Lucanusianus Root on Oxidative Stress, Liver Function and Haematological Markers in Malaria-infected Wistar Mice

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ABSTRACT

Background: Malaria is associated with oxidative stress, haematological alterations, and organ damage. *Costus lucanusianus* is traditionally used in the management of fever, but its antimalarial potential is not well established.

Materials and Methods: The methanol root extract of *C. lucanusianus* was evaluated in *Plasmodium berghei*-infected mice using the 4-day suppressive test. Acute toxicity (Lorke's method), parasitemia suppression, survival time, haematological indices, oxidative stress markers, liver function tests, and histopathology were assessed.

Results: The extract was safe up to 5000 mg/kg. It produced dose-dependent parasite suppression, with 500 mg/kg achieving 50% inhibition, though less effective than chloroquine (62.5%). Treated mice showed prolonged survival, improved antioxidant enzyme activity, and reduced lipid peroxidation. Haematological parameters indicated increased WBC and platelet counts with reduced neutrophils. Liver function markers remained largely stable, while histopathology showed milder hepatic distortion compared to untreated controls.

Conclusion: *Costus lucanusianus* root extract demonstrates significant antimalarial, antioxidant, and immunomodulatory activities, supporting its ethnomedicinal use and potential as a source for new antimalarial agents.

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INTRODUCTION

Malaria is a disease whose pathology is linked to inflammation and oxidative disease. During the blood stage of infection, the level of oxidative stress in plasma is frequently measured by determining the concentration of malondialdehyde (MDA), a lipid peroxide which is formed as a consequence of oxidation of unsaturated lipids and reflects the levels of free radicals in the circulation.[1] The increase in oxidative stress observed in malaria patients infected with either *P. falciparum* or *P. vivax* infections is often coupled with a decrease of anti-oxidant levels which leads to the loss of the homeostatic balance between free radicals and antioxidant capacity that is maintained in healthy tissues.[5]

Oxidative stress is caused by reactive oxygen or nitrogen atoms that have unpaired electrons in their outer shell. They are called reactive oxygen species (ROS) or reactive nitrogen species (RNS) and are commonly produced in cells. These radicals are oxidants that can damage cellular components, but are also involved in essential cellular processes, such as intracellular signaling and the oxidative burst in innate immune cells.[2] Oxidative stress can induce inflammation, since

ROS regulate the inflammatory response in immune cells through the activation of NF-kB, which results in the secretion of inflammatory cytokines.[3] ROS also serves as the first signal for the activation of the inflammasome,[4] further contributing to the inflammatory response.

Given that Plasmodium parasites are sensitive to ROS-mediated damage,[5] it is not surprising that various antimalarial treatments exploit this feature of the parasite to limit its growth within human hosts. For example, Quinolones, including chloroquine and amodiaquine, act by inhibiting the conversion of free heme to haemozoin within the infected erythrocyte, effectively increasing oxidative stress for Plasmodium parasites.[6]

Malaria is usually associated with various degrees of reduced blood counts, and mild to moderate thrombocytopenia is a common association of malaria but it is rarely associated with hemorrhagic manifestations or a component of disseminated intravascular coagulation.[7] Haematological parameters that are often affected include the relative numbers of circulating cell types such as erythrocytes, platelets, granulocytes and lymphocytes, as well as parameters like haemoglobin concentration.[8]

Malaria is known to cause liver and kidney damage especially is severe cases. In fact, it is the first parasitic infection to be clearly associated with glomerular diseases in the tropical region.[9] Hepatic dysfunction and jaundice are common features of severe malaria.[10]

Medicinal plants and natural products have been of scientific interest for their potential role in modulating oxidative stress and inflammatory-related disorders.[11] These resources are abundant in antioxidants, such as flavonoids, polyphenols, and vitamins which can neutralize harmful free radicals and reduce oxidative stress associated with various chronic diseases.[11] The plant of choice, *Costus lucanusianus* was selected as it was reported to possess anti-inflammatory activities.[12]

MATERIALS AND METHODS

Experimental animals

Thirty (30) wistar mice used for this study were 6-week-old wistar mice weighing 19-35 g obtained from the Animal house, Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt. They were housed in plastic cages with saw dust as beddings and were given food and water ad libitum. The animals were allowed to acclimatize for 7 days preceding the experiments. All experimental protocols followed internationally accepted principles for laboratory animal use and care.

Plant material and Authentication

Fresh roots of *Costus lucanusianus* were collected from the forest reserve of University of Port Harcourt, Nigeria (4.91°N, 6.92°E). The plant was identified by Prof. I. Agbagwa of the department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, and a sample was deposited at the University of Port Harcourt Herbarium with reference number UPH/V/1212

Preparation of Methanol extract of Costus lucanusianus root (MECL)

After collection of the plant, the roots were washed, chopped into smaller pieces and shade-dried at room temperature (32 – 35°C) to constant weight over a period of twenty-one (21) days. Bulk of the plant was collected between June 2023 and February 2024. The cold maceration extraction method was used. 500 g of dried *Costus lucanusianus* root was weighed and ground to fine powder and dissolved in 2500mL of methanol inside a 2.5L conical flask. The flask was shaken

vigorously at 30-minute intervals and left to stand for 72 hours at room temperature for effective extraction. The resultant mixture then was filtered with Whatman's No. 1 filter paper and cotton wool. The clear solution obtained was concentrated with rotary evaporator at 45°C under low pressure and later transferred to evaporating dish over a steam bath. The solid dried powder obtained was stored in sterile pre-weighed screw capped bottles and labelled accordingly. The extract was now stored at room temperature.

Acute Toxicity test in Mice

The toxic effect of methanol extract of *Costus lucanusianus* root was determined on wistar mice. Lorke's method was used for the study.[13] It had two phases. Phases 1 required nine animals and divided into three groups. Each group was administered 10, 100 and 1000mg/kg of the extract. The animals were placed under observation for 24 hours to monitor if mortality will occur.

Phase 2: Three animals were used in this phase. The animals were administered higher doses, 1600, 2900 and 5000 mg/kg of the extract respectively. They were then observed for 24 hours for mortality. Then the LD_{50} was calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

In vivo Antimalarial test of Fraction

The anti-malarial activity of methanol extract of *Costus lucanusianus* root was assessed by the classic 4-day suppressive test.[14] Mice were inoculated intraperitoneally (i.p) with blood containing 1 x 10^7 parasitized (CQ-sensitive *P. berghei berghei* NK 65 strain) erythrocytes contained in 0.2 mL inoculum on Day zero (D_0). The treatments commenced 1hour after the infection. Plant extract was solubilized in dimethyl suphoxide (DMSO) and administrated at different concentrations ($1/50^{th}$, $1/25^{th}$ and $1/10^{th}$ mg kg^{-1}/day of the LD_{50})[15] in a dose volume of 0.2 mL. The animals were placed in six groups with five mice in each group. Group 1, 2 and 3 served as the normal, negative and positive control respectively. Group 4-6 served as the treated groups. For the period of treatment (D_1 – D_3), blood samples were collected from tail snips of the mice daily and were made into thin films. The blood films were developed for microscopic examination to monitor their parasite densities. The % suppression (inhibition) of parasitaemia was calculated by comparing the parasitaemia present in negative control with those of treated group.

$$\%\ Parasitemia = \frac{\text{Number of parasitized RBC}}{\text{Total RBC}} \times 100$$

$$\%\ Suppression = \frac{\text{Mean parasitemia of negative control} - \text{Mean parasitemia of treated group}}{\text{Mean parasitemia of negative control}} \times 100$$

$$\text{MST} = \frac{\text{Total number of days mice survived}}{\text{Total number of mice}} \times 100$$

Liver function parameters

Blood plasma for liver function parameters were obtained from the blood samples following centrifugation at 3000 rpm for 5 min. Aspartate transaminase (AST) and Alanine transaminase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP) and Albumin were estimated using standard laboratory kits (Randox Laboratories Limited, Crumlin, County Antrim, BT294QY, United Kingdom), as per manufacturer's instructions.

Haematological assays
Haematological analysis of the blood samples was performed using an automated haematology analyzer (2800 Haematology AutoAnalyzer). Parameters which were evaluated included white blood cell (WBC), red blood cell (RBC), haemoglobin concentration (Hg), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Oxidative stress markers
Catalase activity, superoxide dismutase, glutathione, glutathione peroxidase (GPX) activity and malondialdehyde (MDA) were estimated.

Histopathological examination
The liver specimens from each mouse were immediately stored in 10%v/v formalin in normal saline after gross histological examination and dehydrated using increasing concentrations of isopropyl alcohol (80-100%).

Data Analysis
Data from the study was analysed using GraphPad prism v. 9.02 statistical software. Data was presented as mean±SEM. Significance was considered at $p< 0.05$ using one-way analysis of Variance (ANOVA) followed by tukey's post hoc test.

Ethical Approval
The study was approved by the Research Ethics Committee of the University of Port Harcourt with approval number **UPH/CEREMAD /REC/MM105/080**.

RESULTS
Suppressive effect of Methanol root extract of Costus lucanusianus in *Plasmodium berghei* infested mice

Table 1 showed the Suppressive effect of methanol root extract of *C.lucanusianus* on *Plasmodium berghei* infested mice for day one. The result showed no significant difference between the negative control and the treated groups. Also, there was no significant difference between the positive control (5 mg/kg Chloroquine) and the treated groups. Table 2 showed significant difference when the treated groups were compared with the normal control (NC), negative control and positive control. It was also observed that the significance was dose dependent. As the dose of the extract increased, there was reduction in the parasite load and an increase in % inhibition. Table 3 showed further decrease in the % parasite load of the treated group when compared with the negative control. 500 mg/kg showed greater inhibition (50%) and its % parasite load (10.00±0.30) was close to the positive control (5 mg/kg CQ). The mean survival time (MST) showed an increase in survival time as the doses of the extract increased.

Table 1: Suppressive effect of Methanol root extract of *Costus lucanusianus* on *Plasmodium berghei* infested mice (DAY 1).

GROUPS	Parasite load (%)	% Inhibition	MST
NC	00±00		30.0±0.00
P.berghei	13.50±0.35		6.67±0.88
5 mg/kg CQ	12.00±0.80	11.1	29.00±0.58
100 mg/kg MECL	13.00±0.20[a]	3.7	19.35±0.77
200 mg/kg MECL	12.60±0.10[a]	6.7	21.34±0.88
500 mg/kg MECL	12.50±0.15[a]	7.41	25.01±1.02

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; MST: Mean Survival Time; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 2: Suppressive effect of Methanol root extract of Costus lucanusianus in Plasmodium berghei infested mice (DAY 2).

GROUPS	Parasite load (%)	% Inhibition	MST
NC	0.0±0.0		30.0±0.00
P.berghei	21.50±1.06		6.67±0.88
5 mg/kg CQ	11.00±0.40	48.8	29.00±0.58
100 mg/kg MECL	17.00±0.10[abc]	20.9	19.35±0.77
200 mg/kg MECL	15.00±0.50[abc]	30.2	21.34±0.88
500 mg/kg MECL	15.00±0.30[abc]	30.2	25.01±1.02

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; MST: Mean Survival Time; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 3: Suppressive effect of Methanol root extract of Costus lucanusianus in *Plasmodium berghei* infested mice (DAY 3).

GROUPS	Parasite load (%)	% Inhibition	MST
NC	0.00±0.00		30.0±0.00
P.berghei	20.00±1.56		6.67±0.88
5 mg/kg CQ	7.50±0.25	62.5	29.00±0.58
100 mg/kg MECL	15.00±0.20^{abc}	25	19.35±0.77
200 mg/kg MECL	13.00±0.10^{abc}	35	21.34±0.88
500 mg/kg MECL	10.00±0.30^{ab}	50	25.01±1.02

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; MST: Mean Survival Time; Values represented as mean±SEM; ^{a}p<0.05 when compared with NC; ^{b}p<0.05 when compared with P.berghei; ^{c}p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Effect of Methanol root extract of Costus lucanusianus on liver function, haematological and oxidative stress parameters of wistar mice

Table 4 showed no significant change in the levels of the liver function parameters. In table 5, it was observed that white blood cells significantly ($p<0.05$) increased in the treated groups when compared with the negative control. It was also noticed that the extract did not bring the WBC back to that of normal control (4.55±0.24). Platelet was also observed to significantly ($p<0.05$) change in the treated groups when compared with the negative control. Table 6 showed that haematological parameters were not significantly ($p<0.05$) changed except for neutrophils which was significantly ($p<0.05$) decreased in the treated groups when compared with the negative control.

In table 7, there was no significant ($p<0.05$) difference in the values of Glutathione (GSH) when compared with negative control. However, the treated groups varied significantly ($p<0.05$) when compared with the normal control. There was also a significant ($p<0.05$) difference in the concentration of superoxide dismutase (SOD) between the treated groups and negative control. The positive control (5 mg/kg CQ) group showed a significant ($p<0.05$) difference in SOD when compared with the treated groups. Malondialdehyde (MDA) had no significant ($p<0.05$) change when the treated group was compared with the negative control. However, 500 mg/kg MECL reduced MDA significantly ($p<00.05$) when compared with the positive control (5 mg/kg CQ).

Table 4: Effect of Methanol root extract of Costus lucanusianus on liver function parameters of wistar mice.

GROUPS	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	TP (g/dl)	ALB (g/dl)
NC	28.00±2.00	23.00±2.00	33.50±2.12	64.00±1.41	42.00±1.41
P.berghei	25.50±1.50	22.00±1.00	32.00±1.41	53.00±2.83	34.50±2.12
5 mg/kg CQ	37.50±2.50	25.50±3.50	37.50±0.71	55.50±2.12	36.50±1.50
100 mg/kg MECL	25.50±2.29	18.00±1.22	32.00±3.59	51.50±4.21	34.02±1.87
200 mg/kg MECL	26.50±1.99	15.50±0.73	28.08±2.72	48.00±3.46	31.00±0.96
500 mg/kg MECL	28.50±3.18	19.00±1.85	29.00±3.11	63.50±1.21	42.00±3.12

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; AST: Aspartate transferase; ALT: Alanine transaminase; ALP: Alkaline Phosphatase; TP: Total Protein; ALB: Albumin; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 5: Effect of Methanol root extract of *Costus lucanusianus* on haematological parameters of wistar mice.

GROUPS	PCV (%)	Hb (g/dL)	RBC($\times 10^6/\mu l$)	WBC $\times 10^3/\mu l$)	PLT ($\times 10^3/\mu l$)	MCHC (g/dL)
NC	40.0±1.41	12.10±0.90	7.20±0.28	4.55±0.24[a]	510.0±87.0[a]	29.75±2.57
P.berghei	44.0±2.03	13.35±0.07	8.05±0.85	6.45±0.27[b]	254.0±52.0[b]	30.45±4.31
5 mg/kg CQ	37.0±1.41	11.65±1.45	6.65±0.07	7.95±0.40[c]	148.5±53.5[c]	31.15±4.45
100 mg/kg MECL	42.50±2.50	13.65±0.95	7.00±1.10	11.85±0.45[abc]	714.5±68.5[bc]	31.45±3.15
200 mg/kg MECL	38.50±0.71	11.80±1.40	6.50±0.20	7.15±0.13[a]	561.0±21.9[c]	30.00±4.24
500 mg/kg MECL	42.50±3.53	12.65±1.55	7.80±0.28	7.70±0.21[a]	495.0±63.51[c]	29.00±1.90

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; PCV: Packed Cell Volume; Hb: Hemoglobin; RBC: Red Blood Cells; WBC: White Blood Cells; PLT: Platelets; MCHC: Mean Corpuscular Hemoglobin Concentration; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 6: Effect of Methanol root extract of *Costus lucanusianus* on haematological parameters of wistar mice.

GROUPS	MCH (g/dL)	MCV (g/dL)	Neutrophil (%)	Lymphocytes (%)	Eosinophil (%)	Monocytes (%)
NC	16.85±1.65	56.55±2.15	22.00±1.41[a]	71.50±10.5	1.50±0.05	5.00±0.16
5 mg/kg CQ	17.35±2.05	55.70±1.27	21.00±1.81[b]	72.00±14.4	1.00±0.03	6.50±0.27
P.berghei	16.65±1.85	54.80±0.99	10.50±0.85[c]	83.50±12.50	1.50±0.01	4.50±0.15
100 mg/kg MECL	19.65±1.55	62.60±5.80	3.00±0.10[abc]	94.50±15.00	1.00±0.00	3.02±0.10
200 mg/kg MECL	17.90±2.12	59.85±0.95	3.50±0.50[abc]	92.50±21.21	1.00±0.00	3.01±0.11
500 mg/kg MECL	16.10±1.60	55.60±1.80	5.00±0.83[ab]	91.00±13.00	1.00±0.00	3.03±0.14

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; MCH: Mean Corpuscular Hemoglobin; MCV: Mean Corpuscular Volume; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 7: Effect of Methanol root extract of *Costus lucanusianus* on oxidative stress markers

GROUPS	GSH	GPx	CAT	SOD	MDA
NC	2.69±0.02[a]	0.081±0.0009[a]	1.18±0.11[a]	0.40±0.029[a]	0.35±0.038[a]
P.berghei	2.34±0.10[b]	0.073±0.0052[b]	0.95±0.16[b]	0.22±0.020[b]	0.55±0.021[b]
5 mg/kg CQ	2.09±0.08[c]	0.063±0.0009[c]	0.89±0.015[c]	0.19±0.012[c]	0.58±0.006[c]
100 mg/kg MECL	2.11±0.08[a]	0.062±0.0018[a]	1.43±0.124[bc]	0.41±0.023[bc]	0.41±0.035
200 mg/kg MECL	2.28±0.06[a]	0.072±0.0038	1.52±0.02[bc]	0.35±0.49[bc]	0.40±0.065
500 mg/kg MECL	2.31±0.06[a]	0.075±0.0044	1.70±0.04[abc]	0.40±0.019[bc]	0.36±0.048[c]

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; GSH: Glutathione; GPx: Glutathione peroxidase; CAT: Catalase; SOD: Superoxide Dismutase; MDA: Malondialdehyde; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Histology

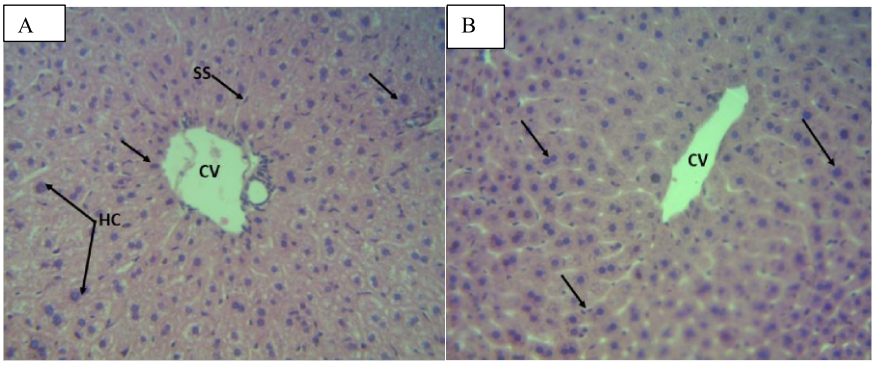

Figure 1: (A) NORMAL CONTROL: Photomicrograph (H&E X400) of the liver showing the centrilobar area of the central vein (CV): visible hepatocytes (HC) with kupfer cells within the sinusoids (SS) draining into the central vein. Livers tissues appear normal (arrows). Diagnosis: Normal liver tissue. (B) POSITIVE CONTROL: Photomicrograph (H&E X400) of the liver architecture showing minimal congestion of the central vein, sinusoids with kupfer cells and hepatocytes (arrows): tissue appears normal Diagnosis: Normal appearance of the liver parenchymal.

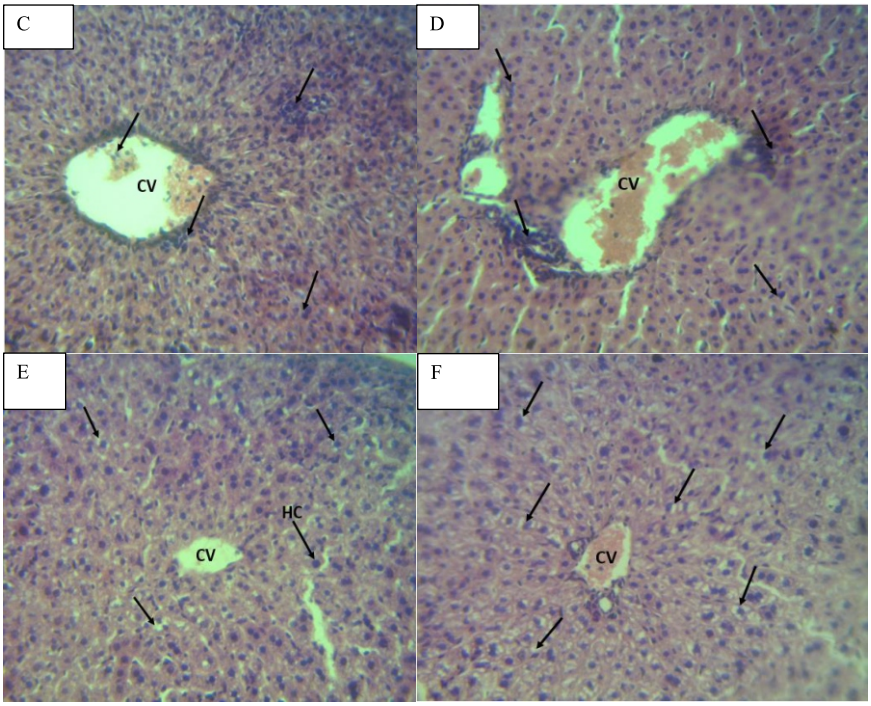

Figure 2: (C) NEGATIVE CONTROL: Photomicrograph (H&E x400) of the liver showing severely diffused mononuclear infiltration within the central vein and surrounding liver parenchymal (arrows) Diagnosis: Severe Inflammation of the liver tissue. (D) METHANOL LOW DOSE: Photomicrograph (H&E X400) of the liver showing mild sinusoidal dilation and mononuclear infiltration around zone 1 of the liver parenchymal (arrows). Diagnosis: mild inflammation of the liver tissue. (E) METHANOL MEDIUM DOSE: Photomicrograph (H&E X400) of the liver with congestion of the central vein with mild glycogen degeneration of the liver parenchymal (arrows). Diagnosis: mild fatty degeneration of the liver cytosol. (F) METHANOL HIGH DOSE: Photomicrograph (H&E X400) of the liver with congestion of the portal vessel with diffused glycogenesis of the liver parenchymal (arrows). Diagnosis: Glycogen degeneration of the liver parenchymal.

DISCUSSION

This study elucidated the effect of methanol extract of Costus lucanusianus root on liver function, haematological and oxidative stress markers. It adopted an in vivo model in order to factor in possible prodrug effect and involvement of the immune system in extermination of infection.[16] The acute toxicity result of the plant extract showed that it was safe to be taken at above 5000 mg/kg. Based on Erhirhie et al.,[17] the plant is practically non-toxic as the dose was within the range of 5000 – 15000 mg/kg.

The study found that different doses of the extract lowered parasite load in the animals and enhanced their survival time in a dose-dependent manner. It is hence concluded that the plant's antiplasmodial activity would be in the early infection stage where malaria's primary attack can be measured.[18] Although, 500 mg/kg of the extract reduced the parasite load significantly at day 3, chloroquine showed a better result and inhibited parasite load by 62.5%. This result is in contrast with Afshar et al[19] who stated that only dichloromethane (DCM) and n-hexane extracts of all aerial root of S. frigida indicated high to moderate anti-malarial potency in comparison with the reference control, while methanol extract of aerial root of S. frigida showed no significant anti-malarial activities. However, Uzor et al.[20] stated that the methanol fraction was the most active fraction of D. edulis.

Hepatic dysfunction and jaundice are common features of severe malaria. In malaria infection, there is usually an increase in ALT, AST, ALP, Total bilirubin, and a decrease in albumin.[10] Table 4 showed that there was no significant change in the levels of the liver function parameters of animals treated with methanol root extract of Costus lucanusianus when compared with the negative control. The result gotten from this study is in contrast with Megabiaw et al[27] which stated that there was increased AST, ALT and ALP of malaria patients before drug treatment. Haematological abnormalities are considered a hallmark of malaria and are reported to be most pronounced in P. falciparum infections. The study revealed that white blood cells and platelet were significantly increased for 100 mg/kg methanol extract (Table 5). Also, neutrophils were significantly reduced for 100, 200 and 500 mg/kg methanol extract (Table 6). Other haematologic parameters were not significantly altered. The result was in contrast with a study that posited that leucopenia was frequently seen in the malaria-infected patients.[21] However, another study agreed with this research that leukocytosis was demonstrated in malaria infested patients.[22] This study aligned with a previous study that reported that malaria induced a reduction in neutrophil levels.[23] The underlying mechanisms include the marginalization of neutrophils to the sites of inflammation, splenic localization, serum lymphotoxic factors, and intercurrent bacterial infections.[23] Studies show that neutrophil numbers may be affected by antimalarials. [24,25] Thus, it explains the much reduction observed in neutrophil levels for the treated groups.

Since malaria is a highly inflammatory and oxidative disease, the incidence of oxidative stress is almost inevitable. Results show that high levels of oxidative stress were found in mice infected with P. berghei, P. yoelii or P. chabaudi,[26] indicating that oxidative stress is a major complication in Plasmodium infections. The result from this study indicated that oxidative stress markers for the treated groups were significantly different from that of the negative control except for GSH and GPx. The stress markers were seen to be restored in the animals administered with the extract. Malondialdehyde level was also seen to be restored to almost normal in the treated groups.
Histopathology of the liver indicated severe

inflammation of the liver cells while the costus treated groups expressed mild to moderate distortion of the liver.

CONCLUSION

The study revealed that methanol extract of C. lucanusianus root has promising antiplasmodial potential as it significantly reduced parasite load. The study also showed that liver function parameters did not significantly change but there was significant change in the levels of platelets, white blood cells and neutrophils. It was also shown that the extract restored oxidative stress markers to normal control.

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Case Reports

Chronic Myeloid Leukaemia in Pregnancy – A Case Report

Dirisu, IM[1] & Ohwotake EI[2]

ABSTRACT

Introduction: Chronic myeloid leukaemia is a Haematological malignancy characterized by the accumulation of monoclonal myeloid cells in peripheral blood and bone marrow. CML in pregnancy presents an intriguing scenario as first line therapy with tyrosine kinase inhibitors (TKI), are contraindicated due to fetal complications. Major presentations include features of hypermetabolism and bleeding complications such as antepartum and postpartum hemorrhages. CML is rare in pregnancy, occurring in about 1 in 75,000 pregnancies with the prevalence in Nigeria not documented.

Case Report: An unbooked 37 year old woman with markedly increased absolute neutrophil count with basophilia who had hysterectomy done on account of severe postpartum haemorrhage prior to a diagnosis of chronic myeloid leukaemia being made. This case report describes the delayed presentation and clinical management of the patient with CML and challenges associated there with.

Conclusion: CML in pregnancy is rare and is associated with several challenges in terms of presentation and management.

Keywords: Chronic Myeloid Leukaemia, Pregnancy, Nigeria

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INTRODUCTION

Chronic myeloid leukaemia is a haematological malignancy characterized by the accumulation of monoclonal myeloid cells in peripheral blood and bone marrow usually with associated infiltration of the spleen by the malignant cells. The diagnosis of CML is rarely difficult and is assisted by the characteristic presence of the Philadelphia (Ph) chromosome. This results from the t(9;22)(q34;q11) translocation between chromosomes 9 and 22 resulting in the chimeric BCR-ABL1 gene. [1] CML is the second most common of the leukaemias in the Niger Delta region of Nigeria, representing 11% of all haematological malignancies [2] with a mortality rate of 13.6%.[3] It occurs in either sex with equal sex distribution. Majority (62.5%) of cases occur between the ages of 30 and 49 years.[2] In up to 50% of cases the diagnosis is made incidentally from a routine blood count. [1]

CML is rare in pregnancy, occurring in about 1 in 75,000 pregnancies.[4] The prevalence of CML in pregnancy in Nigeria is not documented.. However, it is estimated to occur in about 10% of pregnancies.[5] The challenges and complications of CML in pregnancy, such as antepartum and postpartum haemorrhagic phenomenal and

intrauterine foetal growth restrictions, as well as the potential effects of it's treatment on maternal and foetal health, ranging from infertility to foetal malformations, are well documented.[6,7]

CASE REPORT

Mrs OA, a 37 year old secondary school teacher first presented at a general hospital in a remote area with complaints of generalized body weakness of a week duration and bleeding per vaginal of two days duration. She was then an unbooked G3P3+0 lady presenting at 31+2 estimated gestational age (EGA). She was placed on bed rest while some medications were administered to her. No investigation was carried out. On day two on admission she was referred to the Mother and Child Specialist Hospital in the nearby town due to persistent bleeding. There, obstetric ultrasound scan revealed antepartum haemorrhage and liver and splenic spans of 18cm and 22cm respectively. Total white cell count (WBC) was 117,300/uL with a absolute neutrophil count (ANC) of 107,000/uL. Hematocrit (HCT) was 33% while platelet count was 387,500/uL. She was placed on intravenous antibiotics while being worked up for a emergency caesarean section. Patient however, had a spontaneous vaginal delivery while on the operating table in the theater. A live born neonate weighing 1.8kg with an apgar score of 7.8 was delivered. She was discharged third after delivery but represented two weeks later due to postpartum haemorrhage and fever. A Full Blood Count (FBC) done showed a WBC of 240,000/uL, ANC of 236,000/uL and basophil count of 1,100/uL. HCT was 28% while platelet count was 435,000/uL. She was placed on heavier intravenous antibiotics and evacuation of retained products of conception (ERPC) was done with minimal yield. Due to persistent vaginal bleeding and clinical decompensation, an hysterectomy was eventually carried out. Post operative FBC done showed a WBC of 170,000/uL, ANC of 162,00/uL, platelet count of 373,000/uL and HCT of 30%. She was stabilized and later discharged a week later with a referral to see a haematologist.

Patient presented at our facility ten weeks after she was discharged from the Mother and Child Specialist Hospital with complaints of fatigue, malaise, vertigo, headaches, fever, excessive sweating and anorexia. She was acutely ill looking with a temperature of 38.6^{0}C, mildly pale and dehydrated. There were no palpable peripheral lymph nodes. Her abdomen was mildly uniformly distended with a healed pfannenenstiel scar. The liver and spleen were palpably enlarged measuring 8cm and 12cm from the right and left subcostal regions respectively. They were firm and non-tender. She was conscious but lethargic and could barely sit up in bed due to vertigo.

FBC showed a HCT of 30%, WBC 178,000/uL, ANC 168,000/uL, basophil count 1,000/uL, platelet count 324,000/uL. Peripheral blood film revealed markedly increased myeloid cells with a complete spectrum of the granulocytic series, basophilic and adequate platelet. Bone marrow cytology revealed a hypercellular marrow with hyperplastic myelopoiesis and active megakaryopoiesis. Blast cells were less than 5%. Therefore, a diagnosis of CML in chronic phase was made. Patient was commenced on hydration, allopurinol, haematinics and hydroxycarbamide (hydroxyurea) at 20mg/kg (1.5g) once daily. Day five on admission WBC had dropped to 79,000/uL, platelet count was 220,000/uL and HCT was 26%. She was now able to get out of bed without tripping over, appetite was returning, fever had subsided and she felt stronger. She was discharged on request after samples for BCR-ABL 1 transcript was taken.

Two weeks into therapy, there was resolution of all initial presenting complaints, but she had developed neutropenia (ANC 800/uL). Hydroxycarbamide was discontinued and recommenced a week later at 1g daily following resolution of neutropenia. Patient is strongly being encouraged during each clinic check to visit the foundation where the targeted therapeutic agent, Imatib mesylate is freely given. But she is yet to do so due to financial constraints.

DISCUSSION

Chronic myeloid leukaemia, BCR::ABL1 rearrangement positive is a clonal disorder of a pluripotent haemopoietic stem cell. The diagnosis of CML is rarely difficult and is assisted by the characteristic presence of the Philadelphia (Ph) chromosome.[1] However, diagnosis during pregnancy is usually delayed as early symptoms of CML are nonspecific.[6] Up to 50% of cases of CML diagnosis are incidental from a routine blood count. In those cases where the disease presents clinically, symptoms related to hypermetabolism, such as weight loss, lassitude, anorexia or night sweats are seen. Splenomegaly is nearly always present and may be massive. Features of anaemia may include generalized body weakness, shortness of breath and easy fatigability. Some may have features related to thromboembolic events and these are particularly exaggerated in pregnancy.[6,8] Haemorrhagic phenomena such as antepartum and postpartum haemorrhages (as witnessed in this index patient) are a particularly common presenting complaints in pregnancy related CML.[6]

Tyrosine kinase inhibitors (TKIs) are the mainstay of the treatment of CML and several different drugs are now available.[1] However, these are contraindicated in pregnancy due to their teratogenic complications.[8,9,10] The drug of choice for CML in pregnancy is interferon-alpha as studies have assured of it's safety throughout pregnancy.[8,11] Hydroxycarbamide can also be used except in the first trimester as it is associated with IUGR and stillbirth as well as preeclampsia. Leukapharesis has also been documented to effectively reduce highly elevated white cell counts with associated symptoms of hyperviscousity syndrome[8,11]

This patient first presented at two peripheral hospitals where the diagnosis and associated complications of chronic myeloid leukaemia in pregnancy could not be made. Hence appropriate and timely interventions were not instituted leading to inappropriate administration of antibiotics and conduct of hysterectomy. Furthermore, there was delay before patient presented at our facility with consequent clinically deteriorating state. Fortunately, she was still in the early stage of the disease when diagnosis was made and treatment commenced. Of note and in keeping with a previous study on the acceptability and use of molecular targeted therapy [12] is the patient's delay in seeking the use of Imatinib therapy.

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Silent Strangulation: A Case Report of the Unseen Threat of Nuchal Cord Discovered During Caesarean Section.

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ABSTRACT

Background: Nuchal cord, one or more loops of umbilical cord around the foetal neck, is common, with global incidence ranging from 3% to 38%. Although usually benigh, tight multiple loops can lead to significant perinatal issues.

Case presentation: We report a rare case of a 36-year-old primiparous woman induced at 41 weeks for postdatism and reduced foetal movements. Despite normal antenatal findings, persistent variable decelerations during labour prompted emergency caesarean section. Intraoperatively, three tight nuchal cord loops were discovered. The male neonate was delivered with moderate asphyxia (1 minute Apgar score was 5), which was initially concerning. However, he responded remarkably well to our resuscitation efforts, attaining 5-minute and 10-minute Apgar scores of 8 and 10, respectively. The neonate was subsequently observed in Neonatal Intensive Care Unit by the neonatologist for 10 hours.

Discussion/conclusion: The diagnostic limitations of routine antenatal ultrasound in detecting tight nuchal cords, particularly in resource-constrained areas like Nigeria, are highlighted by our index case. This reinforces the importance of individualized clinical assessment, watchful monitoring during labour and maintaining a high index of suspicion when foetal distress persists despite conservative interventions

Keywords: Unremarkable antenatal care, three tight loop of cords, nuchal cords, silent strangulation, caesarean section.

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INTRODUCTION

Nuchal cord, which refers to one or more loops of the umbilical cord wrapped 360° around the neck of the foetus, is a common observation during delivery.[1, 2] The umbilical cord acts as the essential connection between the developing foetus and the placenta, providing crucial nutritive, metabolic, and excretory functions from as early as the fifth week of pregnancy. In addition to its biological function, it represents the deep connection between mother and child.[1] However, issues associated with the cord, especially nuchal cord, where the umbilical cord wraps around the foetus's neck, can have a significant impact on perinatal outcomes. [3]

Nuchal cord is a relatively common obstetric finding, with reported prevalence ranging from approximately 3% to 38% at delivery.[4-7] The incidence of single, double, triple and quadruple loops of cord varies geographically; for example, a Nigerian study noted frequencies of 80.6%, 12.5%, 4.2% and 2.7% respectively.[7] Though often benign, the presence of the cord around the neck can result in intermittent compression during labour, especially as the foetus descends the birth canal.[1] This compression may impair umbilical venous flow, while arterial circulation continues, potentially causing foetal hypovolemia, acidosis, and anaemia, contributing to 5–18% of foetal asphyxia cases and approximately 10% of stillbirths.[8]

Advanced imaging, particularly ultrasonography with Doppler, is the gold standard for antenatal diagnosis. These modalities can assess the number of loops, degree of tightness, and the precise location of cord entanglement.[9] Clinically, the suspicion of a nuchal cord can be raised through transabdominal manual compression tests, in which foetal heart rate decelerations resulting from gentle neck pressure may suggest its existence.[10] Despite advancements in diagnosis, most nuchal cords do not lead to negative outcomes for newborns, and the routine screening during the third trimester is still a matter of debate.[11] The prevailing opinion now leans towards attentive monitoring during labor rather than taking preemptive measures. The occurrence of a tight triple nuchal cord is a rare phenomenon, especially in the literature from Nigeria, and it is linked to a greater risk of complications; however, it does not always require a caesarean section. Following informed written consent from Mrs. A. Z, we present a unique and instructive case of a woman who had induction of labour with persistent variable decelerations and was discovered, during caesarean section, to have tight three loops of nuchal cord, highlighting the unpredictable nature of this condition and the need for individualized and meticulous clinical judgment.

Case presentation

A 36-year-old G5P1+3 delivered a male neonate with three firmly attached loops of nuchal cord through caesarean section (CS) on the 12th February, 2025, at 41weeks gestational age. She had a spontaneous vaginal delivery 3 years ago and 3 premarital terminations of pregnancies with no complication.

The index pregnancy was booked in a private hospital in Benin City, Edo State, Nigeria, at gestational age of 11 weeks and 6 days. She had routine booking investigations done which were essentially normal: There was no abnormality reported in urinalysis; Blood group and Rhesus-B rhesus positive; Genotype-AA; Pack Cell Volume-34%; Retroviral Disease Screening-non-reactive; Hepatitis B and C-non- reactive; Veneral Disease Research Laboratory (VDRL)-non-reactive; fasting Blood sugar level was 80 mg/dl at the booking clinic. During the antenatal period, she received all the routine drugs supplementation: Iron and Folic acid; Monthly intermittent malaria prophylaxis using Sulfadoxine Pyrimethamine; Antitetanus toxoid was also taken according to protocol. Four obstetric ultrasound scans were done at booking, 22, 30 and 36 weeks of gestational age respectively with no abnormal findings reported. The antenatal period remained uneventful with normal maternal and foetal parameters. The assessment at the gestational age of 39 weeks 6 days included a physical examination with Leopold manoeuver revealing a cephalic presentation with the head not engaged and a normal foetal heart tone (FHR) varying between 136 and 142 beats per minute; the estimated foetal

weight was 3.2kg. After detailed discussion with the patient, we mutually agreed on labour induction should spontaneous labour onset fail to occur before the gestational age of 41 weeks and 3 days. On the follow up visit at 40 weeks and 6 days, there was no uterine contractions and the patient reported reduced foetal movements. An ultrasound assessment of biophysical profile showed normal foetal breathing movement, foetal movement, foetal tone and amniotic fluid volume. No cord around the neck was reported and there was normal foetal heart rate of 144 bpm. Following thorough counseling about our clinical findings, the patient and care team agreed on labour induction, with plans for continuous monitoring of both maternal and foetal parameters throughout the induction process. Pre-procedure workups were all normal. Six hours into induction of labour, labour pain had started, and variable deceleration was noted, which persisted despite foetal resuscitation by giving the parturient normal saline, intranasal oxygen and encouraging her to lie down on left lateral position. The parturient and her husband were informed of the findings and decision was reached for emergency caesarean section. A lower uterine segment caesarean section was performed and a live male neonate in cephalic presentation was delivered with 3 loops firm nuchal cord around the neck, Apgar score of 5 at 1 minute, acrocyanosis, and moderately asphyxiated.

Resuscitation was carried out by the neonatologist and anaesthetist, obtaining a 5-minute Apgar score of 8/10 and a 10-minute Apgar score of 10/10. The newborn was transferred to the Neonatal Intensive Care Unit for observation, achieving clinical stability within 10 hours. Investigations revealed no abnormalities. The umbilical cord was centrally inserted on the placenta and measured 76.5 cm. The mother had an uneventful postoperative recovery and she was discharged from the hospital on fifth post operative day and given 2 weeks postnatal clinic appointment.

Figure 1: three tight loops of nuchal cord discovered only at caesarean section

Discussion

The presence of a nuchal cord, defined as one or more loops of the umbilical cord encircling the foetal neck, remains a frequent yet unrecognized intrapartum complication with significant implications for foetal well-being.[12] This case of a 36-year-old primiparous woman delivering a neonate with three tight loops of nuchal cord discovered only at caesarean section emphasizes the complex and yet to be fully understood nature of this condition, particularly in low resource settings, where clinical and basic ultrasonographic assessments may appear deceptively normal, masking a potentially life-threatening condition.

The index patient's antenatal course was uncomplicated, with normal booking investigations, routine supplementation, and serial obstetric ultrasounds failing to detect any cord abnormalities. This agrees with existing literature indicating that antenatal sonographic identification of nuchal cord, while possible, is

not universally reliable, particularly in cases of multiple, tightly wound loops.[13] Despite advances such as Doppler ultrasound and techniques like the gray-scale divot sign[9], accurate diagnosis remains inconsistent and often limited by foetal position, cord thickness, and operator competence.

The decision for labour induction at 41 weeks was clinically justified given the absence of spontaneous onset and reduced foetal movements (a known marker of foetal distress). The persistent variable decelerations observed following induction, which did not resolve with standard foetal intrauterine resuscitative measures, were classical signs of cord compression, as reported in studies by Joshi et al.[8] and Awowole et al.,[7] These decelerations, particularly when unresponsive to conservative management, should prompt early consideration of cord-related pathology, especially in the setting of an otherwise reassuring antenatal profile.

Although existing research generally indicates that a single or even double nuchal cord is not significantly linked to adverse perinatal outcomes, more recent studies suggest that the presence of three or more loops may be associated with a heightened risk of stillbirth or neonatal compromise at birth.[13] In this instance, the intraoperative finding of three tight loops of nuchal cord, a rare phenomenon with a reported incidence of just 4.2% in Nigerian populations,[7] clearly explains the foetal distress encountered. The resultant moderate asphyxia, evidenced by a low initial Apgar score (5 in the 1st minute and acrocyanosis), required prompt neonatal resuscitation. The improvement in Apgar to 8 in the 5th minute and subsequent recovery within 10 hours reinforces the critical importance of timely surgical intervention in mitigating long-term morbidity.

This case also brings to light the limitations of antenatal screening protocols, particularly in low resource settings where reliance on basic ultrasonography may not suffice for detailed cord assessment. It emphasizes the need for individualized clinical judgment, particularly when abnormal intrapartum findings such as non-reassuring foetal heart patterns arise. While tight nuchal cords do not mandate caesarean delivery in all cases, the failure of foetal resuscitation and progressive decelerations in this scenario made surgical delivery the most appropriate course.

Nuchal cords are particularly problematic when they are tightly encircling the foetal neck, as the physiological effects in such cases are conceptually similar to strangulation.[12] Compression of the umbilical cord resulting from a tight nuchal loop can impede blood flow, especially within the thin-walled umbilical vein.

Moreover, the presence of a centrally inserted umbilical cord measuring 76.5 cm, a length well within normal limits, suggests that cord length alone does not necessarily predict adverse outcomes but rather the degree of entanglement and tightness, which can precipitate intermittent hypoxia and ischaemia.

Conclusion: This case adds to the growing body of evidence highlighting the "silent strangulation" caused by undetected nuchal cords. It reiterates the importance of careful intrapartum monitoring, a high index of suspicion for cord complications in the presence of variable decelerations, and prompt decision-making when there is of foetal compromise. As suggested by Mekala et al.[1], while most nuchal cords may be benign, their unpredictability necessitates heightened clinical awareness and preparedness to intervene quickly to ensure optimal neonatal outcomes.

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Diagnostic Delay in Intestinal Malrotation Presenting During Adolescence: A Case Report and Review of Literature

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ABSTRACT

Introduction: Intestinal malrotation refers to any variation in the rotation and fixation of the gastrointestinal tract during development. While the majority of cases present in infancy, particularly with acute obstructive symptoms, a significant number of cases which remain undiagnosed until later in childhood or even adulthood often exhibit vague or nonspecific symptoms, complicating timely diagnosis and management.

Case Report: We report a 10 year old boy presenting with recurrent abdominal pains of 5 years duration associated with recent onset bilous vomiting and constipation. Requested barium meal and follow through showed markedly distended stomach and proximal duodenum that ended blindly. He had exploratory laparotomy with intraoperative finding of malrotation. Ladd's procedure was done and did well postoperatively.

Conclusion: This case report is intended to remind physicians to include intestinal malrotation as a differential diagnosis when an older child presents with recurrent abdominal pain. As early detection may prevent catastrophic bowel ischaemia, chronic midgut volvulus, malnutrition, short bowel and other complications.

Key words: Intestinal malrotation, adolescence, recurrent abdominal pain, Ladd's procedure

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INTRODUCTION

Intestinal malrotation (IM), which encompasses intestinal non-rotation or incomplete rotation, refers to any variation in the rotation and fixation of the gastrointestinal tract during development; this leads to various acute and chronic presentations of disease.[1] The incidence of intestinal malrotation is one in every 200-500 newborns, however symptomatic cases occur in 1 in 6,000 newborns.[2] While the majority of cases present in infancy, particularly with acute obstructive symptoms, a significant number of cases remain undiagnosed until later in childhood or even adulthood. This diagnostic delay can have severe implications, as children presenting after infancy often exhibit vague or nonspecific symptoms, complicating timely diagnosis and management.[3]

In older children, the classic symptoms of bilious vomiting and abdominal distension are less frequently observed, resulting in a delay in diagnosis and poorer outcomes. Arthur *et al*[4] reported an average time to diagnosis of three

months in patients older than one year, with one case experiencing a delay of up to two years due to atypical presentations such as retrosternal pain. The clinical presentation of IM in older children can range from chronic abdominal pain to intermittent symptoms that may last for months or years. Research by Nagdeve *et al*[5] indicated that children over the age of two exhibited significantly increased chronic symptoms compared to their younger counterparts, emphasizing the importance of recognizing these atypical manifestations. Symptoms such as non-bilious vomiting, feeding intolerance, and failure to thrive are common, with many children experiencing recurrent abdominal pain and frequent hospitalizations.[6] Thus, the aim of this case report is to remind physicians to include intestinal malrotation as a differential diagnosis when an older child presents with recurrent abdominal pain, especially when it is associated with vomiting and other gastrointestinal symptoms.

CASE REPORT

A 10 year-old-male admitted into the Children Emergency Unit with a two week history of abdominal pain, vomiting and constipation. The pain was mostly located at the epigastric and umbilical regions, colicky with no known relieving or aggravating factors. Vomiting was projectile, bilious and he vomited about 3 times per day. There was a history of recurrent epigastric pain for the last 5 years, the cause of which remained unidentifiable but received antacids with no relief of the pain. There was no abdominal distension. There were no previous abdominal surgeries or any comorbidity. He was managed for acute exacerbation of peptic ulcer disease at a private hospital for 5 days with no relief of symptoms before referral to our centre.

At presentation, he weighed 27 kg and was dehydrated. His abdomen was scaphoid with no areas of tenderness. There was no organomegaly and his bowel sounds were normoactive. His rectum was collapsed but contained formed stool. Complete blood count showed Haemoglobin concentration of 12 g/dl with normal counts of white blood cells and platelets. Serum electrolytes showed hypokalemia. Abdominal X-Ray on presentation showed double bubble sign. He was resuscitated and hypokalemia was corrected. Requested barium meal and follow through showed markedly distended stomach and proximal duodenum that ended blindly. He was then worked up for exploratory laparotomy on suspicion of duodenal web.

The findings at surgery were: Markedly distended stomach and proximal duodenum with a 360^{o} torsion of the midgut around the superior mesenteric vessels. There were also Ladd's bands from ascending colon to the right lateral wall of the peritoneal cavity. Ladd's procedure was then performed. He had an uneventful post operative recovery period and was discharged home after one week. He has been followed up for 3 months post operatively with no recurrence of the symptoms.

DISCUSSION

Cases of intestinal malrotation presenting during adolescence poses a significant challenge as it is not usually considered as the likely diagnosis based on clinical grounds. These patients often present with symptoms that are mistaken for irritable bowel syndrome, peptic ulcer disease, biliary and pancreatic disease, and psychiatric disorders.[7] Symptoms of intestinal malrotation are linked to the anatomical anomalies associated with it and two distinct patterns of presentation in older children and adults have been reported in the literature: acute and chronic.[8] The chronic presentation is more common. This is characterised by intermittent crampy abdominal

pain, bloating, nausea and vomiting over several months or years which underlines the need for a high index of suspicion of midgut malrotation in older children as the cause of these intermittent and varying abdominal symptomatology.[8] Diagnostic delays are common in this group of patients because of the non-specific nature of the presentations. The pathophysiology of these chronic symptoms may relate to the compression effect of Ladd's bands running from the caecum and ascending colon to the right abdominal wall.[8]

Acute presentation is usually characterized by features of acute bowel obstruction with or without chronicity of symptoms. In cases of acute presentation in the older child or adult with malrotation, midgut volvulus is the most common cause of the bowel obstruction.[9] This was the case in our patient who presented with acute symptoms of colicky abdominal pains and vomiting but had been having chronic symptoms of recurrent abdominal pains for 5 years prior to the acute symptoms.

The diagnosis of malrotation can be made by radiographic studies. In the absence of volvulus of the midgut, a plain x-ray of the abdomen is of little diagnostic value. The absence of cecal gas shadow or the localization of small intestinal loops predominately in the right side should arouse the suspicion of malrotation.[10] The standard upper gastrointestinal series is considered the gold standard for diagnosis and may show a vertical duodenum that does not cross the midline. The entire small bowel is found in the right half of the abdomen. The accuracy of the upper gastrointestinal series is reported to be over 80%.[10] However, in the index case, the findings on upper GI series done were not typical of malrotation syndrome, rather the features were in keeping with a duodenal obstruction. This is probably because the patient had a complete obstruction from the 360o torsion of the midgut around the superior mesenteric vessels.

Malrotation is increasingly being diagnosed with Computed Tomography scan by the anatomic location of a right-sided small bowel, a left-sided colon, an abnormal relationship of the superior mesenteric vessels, and aplasia of the uncinate process.[11] Similarly, abdominal colour Doppler Ultrasound scan may reveal malposition of the SMA, raising the suspicion of gut malrotation with or without the abnormal location of the hollow viscus.[12]

The classic treatment for malrotation is the Ladd's procedure. This involves de-rotation of the bowel if torsion is present, division of the peritoneal attachments lying across the duodenum from cecum to right upper quadrant, widening of the base of the mesentery, appendectomy, taking down the ligament of Treitz, straightening the duodenum to the right and finally, returning the bowel to a position of non-rotation with the cecum placed into the left upper quadrant.[13] Published reports for the laparoscopic Ladd procedure are now appearing in literature as well Laparoscopic Ladd's procedure in asymptomatic patients which confers a shorter time to tolerating feeds, shorter length of stay, and a decreased rate of bowel obstruction and other complications. It may, however, carry a higher risk of postoperative volvulus.[14] Diagnostic laparoscopy is recommended in asymptomatic patients with high suspicion where the diagnosis is not certain after upper gastrointestinal imaging.[15] Our patient had open Ladd's procedure with a good postoperative outcome and has remained asymptomatic after a 3 month follow-up period.

Malrotation of the intestine, though commonly seen in infancy, is a condition that should be

suspected in older children when the patient present with prolonged and recurrent abdominal pain and vomiting. There is a need to have a high index of suspicion for the condition and the use of appropriate imaging modality could help clinch the diagnosis. Early suspicion and detection by the paediatrician and paediatric surgeon may prevent catastrophic bowel ischaemia, chronic midgut volvulus, malnutrition, short bowel and other complications. Complete resolution of acute obstruction or chronic abdominal pain is the result of a high index of suspicion for malrotation, appropriate diagnostic studies, and aggressive definitive treatment.

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Gilles De La Tourette syndrome in one of a set of phenotypically identical twins- a case report with literature review.

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ABSTRACT

Introduction: Gilles de la Tourette's syndrome (GTS) or Tourette's syndrome (TS) is a neurodevelopmental/ neuropsychiatric movement disorder that is characterised by one or more vocal/phonic tics and multiple motor tics. It has a childhood onset and a diagnosis is usually made when symptoms have been present for greater than a year.

Case presentation: We report a 24year old female, one of a pair of phenotypically identical twins who presented with history of verbal and motor tics with associated history of social difficulties and obsessive compulsory disorder. Symptoms were first noticed at the age of nine. There is no known family history of tics and none of these symptoms were present in her twin sister.

Discussion: Aetiopathogenesis of GTS include genetic factors as well as environmental factors such as infections, neuro-immunological factors, and prenatal/ peri-natal difficulties. A younger age of onset is associated with a greater severity of symptoms, while females with GTS are less likely to undergo remission of tics during adolescence, and may experience greater functional interference from tics than males

Conclusion: Persons with GTS may experience learning and cognitive impairments, hence patients presenting with tics should be adequately evaluated for this condition as this will help bridge the gap in delay of diagnosis and management.

Key words: Tourette Syndrome, Tics, Neuropsychiatry, Neurodevelopmental, Phenotypically Identical Twins.

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INTRODUCTION

Gilles de la Tourette's syndrome (GTS) or Tourette's syndrome (TS) is a neurodevelopmental/ neuropsychiatric movement disorder that is characterised by multiple motor and one or more vocal/phonic tics. It has a childhood onset and a diagnosis is usually made when symptoms have been present for greater than a year.[1-3] The condition is named after a French doctor, Georges Gilles de la Tourette, who in an 1884 article described several movement disorders that he believed were similar to each other but different from chorea. In another article in 1885, he further identified 9

subjects who suffered from a disorder distinguished by involuntary movements, strange uncontrollable sounds, echolalia, echopraxia, and coprolalia.[4]

Tics describe brief, sudden and repetitive sounds or movements which are similar to voluntary actions and belong to the spectrum of hyperkinetic movement disorders. Tics also include more complex repetitive behaviours such as echolalia, palilalia, and coprolalia.[5] Tics have been defined as involuntary, recurrent, sudden, rapid, non-rhythmic movements (motor tics) or vocalizations (vocal/phonic tics).[6] Tics are the most common type of movement disorder in children and may occur in bouts up to many times in a single day. The prevalence may range from 1-29% of the population based on the diagnostic criteria, study population, study design and methodology. Some studies give a range of 0.3-1%.[7,8] Tics are often preceded by a subjective unpleasant feeling of 'inner tension of wanting to move' which is temporarily relieved by the appearance of tics. The presence of these sensory symptoms or 'premonitory urges' to tic is important in differentiating tics from other repetitive behaviours such as functional jerks, myoclonus, mannerisms and stereotypies.[6]

The diagnosis of GTS is often straight forward, however the condition is usually not recognised with many patients not correctly diagnosed for many years after the onset of symptoms. Delayed access to standard treatment for the tics and comorbidities such as obsessive-compulsive disorder (OCD) and attention deficit/ hyperactivity disorder (ADHD) may negatively impact quality of life and hamper psychosocial development. Hence, early recognition of tics as well as neuropsychiatric comorbidities are mandatory in the treatment of these patients.[8-12]

GTS is believed to be inherited in most cases, however the genetic mechanisms are complex. It is believed that brain development is affected by general neurodevelopmental genes following which specific GTS gene/genes further affect the phenotype. Other aetiopathogenetic postulations may include environmental factors such as infections, prenatal and peri-natal difficulties including hypoxia/ischaemia, androgen influences, exposure to heat and fatigue, maternal smoking and neuro-immunological factors.[3,13,14]

GTS is a complex heterogenous disease and several studies have demonstrated genetics in its aetiology and its comorbidity with disorders such as ADHD, autism spectrum disorder (ASD) and OCD, however the results have not been consistent. The phenotypic expression in GTS is affected by environmental factors and immune responses. GTS has a complex inheritance pattern in which several genes and loci have been correlated with it such as the Slit and Trk-like 1 (SLITRK 1) and histidine decarboxylase (HDC) which have been identified in Genome-wide linkage studies (GWLSs). Copy number variations are considered another significant source of mutation in GTS and may result from chromosomal deletions or duplications due to polymorphisms.[13-15]

Studies have demonstrated that GTS is familial. A twin study by Price et al. comprised of 43 pairs of same-sex twins, in which at least one co-twin had GTS, 30 pairs were likely monozygotic and 13 were likely dizygotic. Concordances for TS were 53% for monozygotic and 8% for dizygotic pairs. These concordances were consistent with genetic aetiology. Considering that only 53% of the monozygotic twins were fully concordant indicates other nongenetic factors affect expression of GTS. The study was inconclusive on whether some monozygotic twins with concordant co-twins are etiologically different

from those who are discordant. Segregation analysis studies in affected families have shown that GTS is manifested in an autosomal dominant pattern with variable phenotypes, including chronic tic disorders, and OCD. More recent studies indicate polygenic or oligogentic inheritance models.[13,16,17]

Neuroanatomical and brain circuitry have been implicated in GTS with most evidence being that of cortical thinning and a reduction in the size of the caudate nucleus.[3] It is generally believed that patients with GTS have cortical–striatal–thalamic–cortical (CSTC) circuits dysfunction with specific basal ganglia dysregulation. Matrisomes which are subsets of striatal neurones are thought to become abnormally active in inappropriate contexts, resulting in the disinhibition of thalamo-cortical projections that in turn lead to tics. Activity leading to stereotyped repetition of behaviour is inappropriately reinforced by activity-dependent dopamine.[7,18]

Worbe et al. in a study on distinct structural changes which underpin clinical phenotypes in patients with GTS demonstrated that these patients had reduced cortical thickness in their premotor, motor, prefrontal and lateral orbitofrontal cortical areas. The Yale Global Tic Severity Scale was used to assess the severity of tics and these correlated negatively with cortical thinning in these regions, as well as in temporal and parietal cortices. The pattern of cortical thinning was found to differ among the clinical subgroups of patients. Cortical thinning was mostly found in primary motor regions in patients with simple tics, while the thinning extended into larger prefrontal, premotor and parietal regions in patients with simple and complex tics. Patients with associated obsessive–compulsive disorders had reduced cortical thickness in the anterior cingulate cortex with altered hippocampal morphology.[19] GTS is a unique condition that is usually under-recognized and/ or misdiagnosed. There is paucity of reports of this condition especially in sub-Saharan Africa, hence we present this case presentation with discussion.

CASE REPORT

A case of a 24- year old female, one of a phenotypically identical set of twins, who presented at our neurology clinic with history of repetitive abnormal production of sounds and occasional utterances from the age of 9 years (15 years duration). There was associated history of abnormal body movements over which she had no control. Abnormal sounds were said to range from incomprehensible sounds to repetition of nearby conversations. Mother claimed these symptoms were present at the age of 3 years but were mild. The production of sounds occurs at any time of the day but were worse when she was angry or hungry. Abnormal movements involved all limbs but were more associated with the upper limbs and were small range movements. She had also noted facial grimacing. The sounds and movements were said to resolve spontaneously. There was no history suggestive of seizures or tremors nor history suggestive of head injury prior to onset of symptoms. She gradually learnt to control her symptoms over the years by staying in a quiet environment. Attempts to contain the movements or sounds made her uncomfortable, however she was relieved after performing the movements or utterances.

Symptoms worsened over the years making her to seek treatment at different health facilities with psychiatrists and neurologists, however the treatments were not satisfactory. There was history of sleep disturbances, patient said she was not happy with state of her health status. She couldn't understand how she had these symptoms and her phenotypically identical twin sister had

none. Furthermore, there was no family history of similar symptoms. She had been high performing in primary and secondary school but finished her Ordinary National Diploma (OND) with a third class. While at the tertiary institution, she found herself reluctant to go for classes due to her symptoms. Recently she had learnt to control some of the symptoms to an extent. She was placed on Tab Haloperidol 5mg daily in the past but currently on Tab Olanzepine 5mg daily.

Her blood investigations did not reveal any abnormalties. A brain computed tomography (CT) scan done in the past was normal and a brain magnetic resonance image (MRI) requested for was normal. A diagnosis of Gills de la Tourette's syndrome was made based on her motor and vocal tics. She was counselled along with family members on the diagnosis. She was maintained on Tab Olanzepine 2.5mg daily, was subsequently referred to the psychiatrist for co-management. She is currently on follow up and has been stable.

DISCUSSION

Psychopathology of GTS

GTS is described as a neurodevelopmental disorder with childhood-onset characterized by tics that are usually associated with psychiatric co-morbidities.[19] Enhanced structural connectivity in the white matter tracts linking the thalamus and striatum with cortical structures, including primary motor cortex, primary somatosensory cortex, and supplementary motor area have been demonstrated in patients with GTS and was positively associated with increased motor tic severity.[19,20] In childhood and adolescence, the ratio of boys to girls is typically 4:1, but in adulthood, the ratio of males to females is closer to parity. Hence, this childhood gender bias for boys is attenuated in adulthood. This suggests that females with GTS are less likely to undergo remission of tics during adolescence, and may experience greater functional interference from tics than males. It has been demonstrated that females, when compared to males with GTS, have increased connectivity in CSTC pathways which may result in increased tic severity.[19-21]

GTS is associated with a variety of mental illnesses including OCD, ADHD, sleep abnormalities, learning difficulties, or other behavioural problems. The mechanism by which these other neurological conditions are linked to GTS remains unclear. These comorbid conditions are more likely to cause harm than the tics and will need treatment. ADHD occurs in about 50–75% of children with GTS, this suggests that both may share a common pathophysiology in the basal ganglia circuitry.[12] Anxiety, mood disorders, and other emotional symptoms have long been described in patients with TS or ADHD. Recently, there is increased awareness of the clinical and scientific significance of TS or ADHD combined with anxiety, mood, emotional and behavioural disorders. The occurence of mood and anxiety disorders being high among these patients makes them more susceptible to poor school performance, and delayed socio-psychological development.[13,22]

Clinical features/ Diagnosis

A diagnosis of GTS requires the presence of multiple motor tics such as blinking, mouth pouting, mouth opening, head nodding, and one or more vocal tics which include throat clearing, sniffing, and coughing to be present for greater than a year. The frequently reported mean age of onset of GTS is 7 years, but may range from 2-21 years. The onset of phonic tics usually starts at 11 years. Tics may be classified as simple or complex and may have associated premonitory sensations.

Characteristic features of GTS also include palilalia (repetition of what oneself says), Coprolalia (the involuntary and inappropriate use of obscenities), echolalia (repetition of what others say), echopraxia (repetition of someone else's movements) or echomimia (repetition of someone else's facial expressions) occur in patients usually starting from the age of 15 years, however a younger age of onset is associated with a more severe GTS. Coprolalia occurs in 10-15% of patients, however echophenomena (echolalia, echomimia and echopraxia) and palilalia are fairly common and very characteristic.[23,24]

Tic symptoms, the hallmark of Tourette's syndrome (TS), may simply be fragments of innate behaviour. Hence, the sensory urges that precede tics may explain some of the normal internal cues that are intimately involved in the behavioural sequences. The fractal characteristics of tics which occur over time may be responsible for the waxing and waning course of these disorders. Although tics markedly decline during adolescence, GTS may be associated with emotional, social, and academic difficulties in early adulthood. Long-term adaptive outcomes of individuals with GTS may likely be influenced by co-morbid conditions like OCD and ADHD.[25] Affective disorders are common in patients with GTS, with a lifetime risk of 10%, and a prevalence of 1.8–8.9%. Depressive symptomatology was found to occur in between 13% and 76% of cases of GTS seen at specialist clinics.[26] Other issues associated with tics are those of orderliness, symmetry, counting, repetitive checking, aggression, inappropriate sexual behaviours and religiousity.[1,25]

Management

Symptom reduction, management of precipitants and comorbidities is the primary aim of management of GTS. Pharmacological management mainly involves antipsychotics (haloperidol, risperidone, aripiprazole, quetiapine, pimozide, etc.), benzodiazepine (clonazepam, etc.) and alpha agonists (clonidine, guanfacin). The typical antipsychotics (dopamine receptor antagonists) remain the drugs of choice for the treatment of tics with marked improvement in symptoms. GTS patients may also see improvements in their tics with drugs that have partial dopamine receptor agonist activity such as the new generation of antipsychotics.[1,27]

The presence of OCD as a comorbid condition in GTS may require the use of Selective Serotonin Reuptake Inhibitors (SSRI). Other medications that may be used include oxcarbazepine, tetrahydrocannabinol, atomoxetine, and botulinum toxin. Deep brain stimulation may also be used as a means of treatment. Behavioral management primarily includes desensitization techniques, habit reversal, and relaxation exercises.[1,27]

CONCLUSION

GTS is a heterogenous disease with a combination of varied neurological and psychiatric symptoms. Patients presenting with tics should be adequately evaluated for this condition as this will help bridge the gap in delay of diagnosis and management. Learning and cognitive impairments should be identified early to assist with academic progress and social adjustments.

Author's contribution: OEM is responsible for the conceptualization of the case report and literature review, OEM, AOW, OBN and AEB developed, read, and approved the manuscript.

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Uniportal Video Assisted Thoracoscopic Bullectomy for Recurrent Spontaneous Pneumothorax – A first experience case report in a private hospital

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ABSTRACT

Introduction: Spontaneous pneumothorax has several causes; one important cause is lung bullae. Bullae classically occur at the apices of the lungs, and can rupture causing pneumothorax. Resection of these bullae is indicated to prevent recurrence.

Case report: A 25 years old man presented with recurrent breathlessness and cough. Clinical and radiological evaluation revealed a left pneumothorax, for which he had a chest tube insertion. A chest CT scan revealed bilateral lung bullae. He had a repeat pneumothorax after 2 weeks necessitating a repeat chest tube insertion and bullectomy. Surgery was done using the uniportal video assisted thoracoscopic approach which is a relatively novel approach to our institution. He was discharged after 4 days on admission.

Discussion: The clinical presentation of ruptured bulla causing pneumothorax are typical, as our patient was a young man, with sudden symptoms. Uniportal VATS gave the expected advantage over open surgery, as he was discharged home after 4 days, pain was less and cosmetic appeal of the scar was better.

Conclusion: Spontaneous pneumothorax from lung bullae can be safely resected surgically by the uniportal VATS approach in our environment

Keywords: Pneumothorax, Lung bullae, Bullectomy, Uniportal Video-Assisted Thoracoscopic Surgery

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INTRODUCTION

Spontaneous pneumothorax is caused by a variety of pathologies. An important pathology is lung bulla, which gradually increase in size over many years.1 Lung bullae can be unilateral or bilateral. As much as video assisted thoracoscopic surgery (VATS) skills are useful, they requires a learning curve for mastery just like any other motor skill[2]. The usual pathway of skill progression is from open thoracotomy, to three-ports VATS, [3] two-ports VATS, [4] and then to uniportal VATS.[5] Diagnostic procedures and

simple therapeutic cases[6] are usually chosen for such transition in the early stages of the skills curve. Bullectomy is one of the procedures in the learning curve of VATS before advancement to more complex cases.

We report our first experience with uniportal VATS bullectomy in a young man who presented with clinical features of recurrent left sided pneumothorax who eventually went on to have uniportal vats bullectomy following two previous tube thoracostomies in a center where uniportal surgery was not a routine process.

Case-report

A 25-year-old man presented three years ago, with a one day history of sudden left sided chest pain, cough, and difficulty with breathing. Chest pain was of moderate severity, worsened with breathing, but not associated with exertion, was non radiating and there was no significant relieving factor. Cough was dry and breathlessness was of sudden onset and at rest. There was no fever, no weight loss, no preceding history of chest trauma, no drenching night sweats, and no previous history of having chronic cough, or contact with any patient coughing chronically. There was no history of cigarette smoking. He was an office worker, with no history suggestive of exposure to industrial fumes or dust.

Physical examination revealed a young man of average height, he was not pale, afebrile, acyanosed, saturating 95% in room air. Respiratory rate was 30cpm, with right tracheal deviation, reduced left chest expansion, hyperresonant percussion notes and reduced air entry on the left side.

A clinical diagnosis of left tension pneumothorax was made. He came with a chest Xray from the referring hospital which showed hyperlucent left pleural space with absent lung markings and separation of the visceral from the parietal pleura, in keeping with the left pneumothorax (Fig 1). Informed consent was taken, and an emergency left chest tube was inserted, with relief of symptoms. He subsequently had a chest CT scan which revealed expanded left lung, with the chest tube in-situ, but with bilateral apical lung bullae (Fig 2) (Fig 3)

Following clinical improvement and left lung re-expansion, the chest tube was removed, and he was counselled on the need for surgical excision of the bullae, he however wanted some time to decide about surgery.

Two weeks following discharge from hospital, he represented with similar symptoms. A left chest tube was reinserted, and he was again counselled on the need for bullectomy, this time around he consented.

Preoperative laboratory investigations were all within normal limits. Informed consent was taken. Surgery was done under general anaesthesia with one-lung ventilation via a double lumen endotracheal tube, with routine invasive and non-invasive monitoring. He was placed in the right lateral decubitus position, the left hemithorax was prepared routinely and sterile drapes applied, and setup for VATS was made. A 6 cm incision was made in the left 5th left intercostal space, in the mid axillary line. The incision was developed into the left pleural space, the left lung was collapsed, a 10 mm, 30 degrees telescope was inserted into the pleural space to examine the space and check for adhesions. Adhesions were minimal, then a wound protector was placed in the wound (Fig 4) and the telescope reintroduced. Intraoperative findings were flimsy pleural adhesions and left upper lobe apical bullae (Fig 5), normal mediastinal and diaphragmatic pleura. The bulla was attached to the apex of the left lung,

it measured about 10cm in diameter. Adhesiolysis was done with blunt dissection, and use of energy devices as needed. A bullectomy was done with a 30mm EndoGIA stapler (Fig 6), with two reloads. An air leak test was done and the lung was re-expanded. A single size 24 chest tube was placed, and the chest was closed in layers following routine protocol. There was mild air leak which stopped on the second post operative day, and the chest tube was removed on the third post operative day. Post extubation chest Xray showed complete lung expansion (Fig 7), and he was discharged home on the 4th day after surgery.

Follow-up chest CT scan at 30 days (Fig 8) post op were satisfactory. Scar was also satisfactory (Fig 9) .

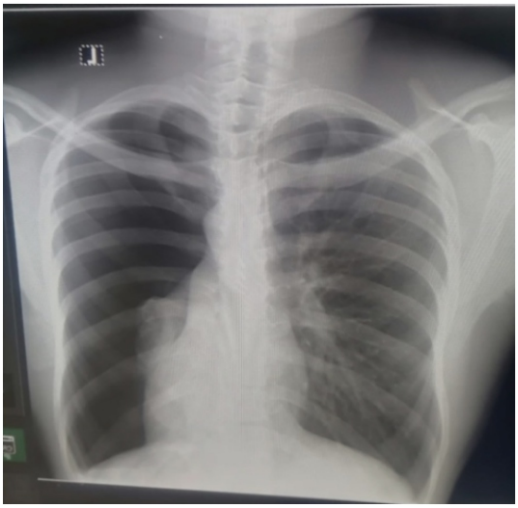

Figure 1: Left pneumothorax

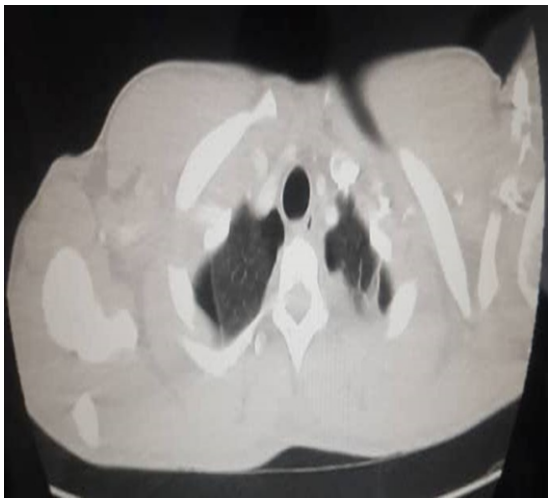

Figure 2: Preop chest CT scan axial showing bilateral apical bullae

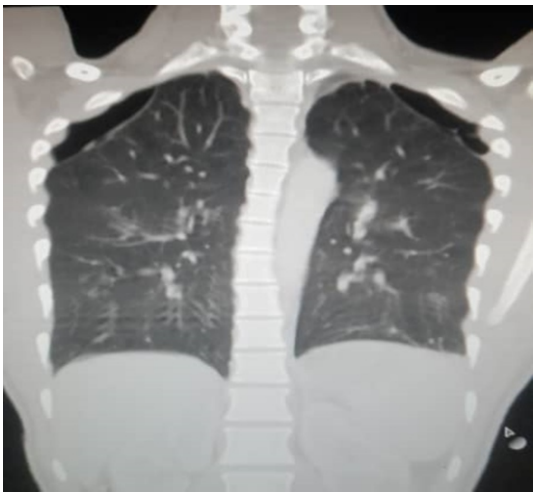

Figure 3: Preop chest CT scan coronal showing bilateral apical bullae

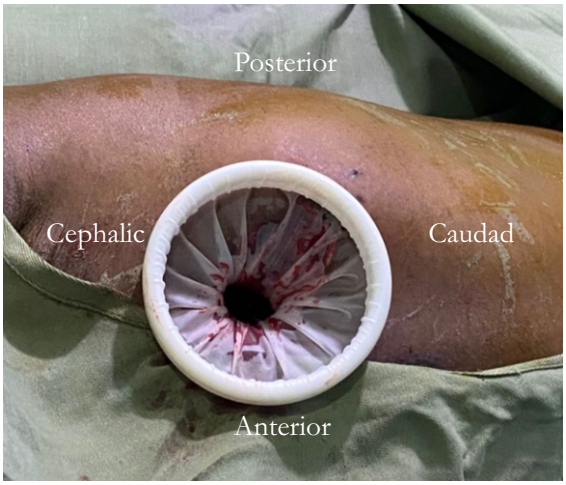

Figure 4: wound protector in-situ

Figure 5: Bulla at apex of left lung

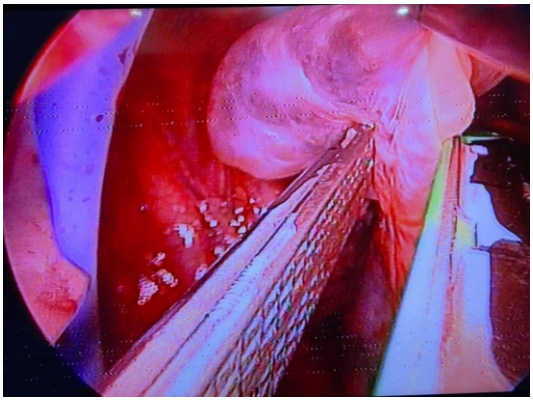

Figure 6: EndoGIA stapler in place at base of bulla

Figure 7: Post-op chest xray showing fully expanded left lung

Figure 8: Post op CT scan post left bullectomy reveals good apex of left lung

Figure 9: Scar at 30 days

DISCUSSION

Pneumothorax is the presence of air in the pleural cavity. It is a common pathology in surgical practice.[7] Spectrum of the disease can range from small pneumothorax without symptoms, to massive with life threatening features of hypotension, cyanosis in tension pneumothorax.

Incidence has been calculated to range from 5.8-16.7 cases per 100,000 persons per year depending on the type of pneumothorax, sex and age.[8] Primary spontaneous pneumothorax is commoner in the younger age group between 20-40 years while secondary spontaneous is commoner in elderly age group 60-70 years.[9] It is more common in men.

There are several causes of pneumothorax. They can be traumatic, iatrogenic, spontaneous or catamenial, and spontaneous pneumothorax is further divided into primary or secondary.[10] In this discussion and in relation to the case presented, this is a case of spontaneous pneumothorax, the primary spontaneous type caused by bullae. Bullae are defined as air filled sacs in the lungs that are more than 1cm in diameter.[11] They are not always symptomatic, as a study in cadavers showed that about one third (33.8%) of the population had small bullae in the lungs.[12] The true incidence in the living population may be under estimated. When bullae rupture, they present with breathlessness and acute pain like in this patient. Secondary spontaneous pneumothorax present more insidiously with other pulmonary symptoms before the respiratory difficulty sets in.

In this index case, there was recurrent sudden symptom of chest pain, breathlessness warranting him coming to the hospital a second time within 2 weeks for treatment. Presentation in our case is typical to the usual presentation of ruptured bulla with pneumothorax. However, there was no pulsus paradoxus, hypotension or elevated jugular venous pressure. We think this is because he was aware of his condition following the previous episode of pneumothorax , so he presented to the hospital early, and had quick intervention. Chest x-ray findings were in keeping with pneumothorax, and was sufficient for initial chest tube insertion. However, a CT scan was requested because the x-ray showed features that made us suspect that there were underlining pathologies most likely bullae which were eventually confirmed.

Treatment for small asymptomatic pneumothorax can be active watchful non-operative follow-up, then drainage for larger cases followed by treatment of any diagnosed underlying cause. Drainage can be by needle aspiration in small symptomatic cases or chest tube insertion in larger pneumothoraxes.

Treatment of underlying cause of pneumothorax and pleurodesis is indicated for first episode of pneumothorax in some group of high-risk patients. These high-risk patients are those in which recurrence is associated with significant risk , examples are airline pilots, scuba divers, people residing in remote places without access to quick surgical care, also those that have contralateral lung disease.[13]

Our patient had an obvious ruptured bullae and it was bilateral, we had to operate to prevent a third occurrence of pneumothorax.

Thoracotomy with resection and suture ligation of the bulla was the previous surgical option for bullectomy. This was associated with significant morbidity. However, with advancement of video assisted thoracoscopy surgery, surgeries are now done with minimally invasive techniques[1,7].
Resection of a lung bulla is relatively a simple

procedure for many thoracic surgeons, and is usually one of the first therapeutic procedures most thoracic surgeon achieve via VATS approach. A uniportal technique is usually acceptable, however, two ports or three ports can be used based on surgeon's experience. In this index case, we were able to achieve our goal with a uniportal technique. It is important to ensure that the staple line is placed on healthy lung tissue to avoid air leak.

CONCLUSION

Spontaneous pneumothorax from lung bullae can be safely resected by the uniportal VATS approach in our environment

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