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From Response to Resilience: Sustaining Vigilance after Ethiopia's first Marburg Virus Disease Outbreak

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EDITORIAL

The recent outbreak of Marburg virus disease (MVD) in southern Ethiopia marks a critical milestone in the country's public health history, confirming the persistent threat of filoviruses in the region (1). Reported by the Ministry of Health in November 2025, the outbreak centered in the Jinka, Malle, and Dasenech districts, resulting in 14 laboratory-confirmed cases and a high case fatality rate of 64.3%. On 26 January 2026, Ethiopia officially declared the end of the outbreak following two consecutive incubation periods (42 days) without new cases and completion of safe and dignified burial procedures for the last confirmed patient. During the response, approximately 3,800 laboratory tests were conducted, and 857 contacts were identified and monitored. Ethiopia has since entered a 90-day enhanced surveillance phase to ensure early detection of any resurgence (2,3). This outbreak, although successfully contained, confirms the presence of ecological and epidemiological conditions favorable for *filovirus* transmission and underscores the importance of sustained preparedness (4).

Marburg virus is an enveloped, filamentous, negative-sense, single-stranded RNA virus (5) belonging to the family *Filoviridae* and genus Marburgvirus. It causes severe viral hemorrhagic fever characterized by the abrupt onset of fever, headache, myalgia, and gastrointestinal symptoms, followed by hemorrhage, shock, and multi-organ dysfunction in severe cases (6–9). The incubation period ranges from 2 to 21 days. Fatal outcomes typically occur within 8–9 days after symptom onset due to systemic viral replication, immune dysregulation, vascular damage, and multi-organ failure (8–10).

MVD is a severe zoonotic viral hemorrhagic fever caused by the Marburg virus, primarily hosted by the Egyptian fruit bat (*Rousettus aegyptiacus*) (11-13). Transmission to humans occurs through exposure to bat-inhabited caves or direct contact with infected bodily fluids (6, 8). Since its first identification in 1967 in Germany (Marburg and Frankfurt) and Serbia (7-9), MVD outbreaks have occurred sporadically across Africa, including Angola, Uganda, Ghana, Tanzania, Rwanda, and Equatorial Guinea (6, 8). Previous outbreaks across Africa have demonstrated the high fatality of the disease, with case fatality rates (CFR) ranging from 20% to 90% (6–8, 14). Ethiopian outbreak (2025–2026) represents a documented expansion of filovirus risk zones, particularly in areas like the Omo Valley, where cave ecosystems support significant bat populations. With no licensed antivirals or vaccines currently available, management remains strictly supportive, making early diagnosis via RT-PCR and rapid isolation the only effective

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tools for survival and containment (1, 15-17).

The successful response demonstrated Ethiopia's coordinated national capacity; however, the transition from reactive response to long-term resilience is essential. To mitigate the risk of future spillovers, Ethiopia must prioritize the decentralization of molecular diagnostic capacity to regional laboratories and establish ecological surveillance of bat reservoirs. Institutionalizing rapid response teams and strengthening cross-border collaboration under a One Health framework, integrating human, animal, and environmental health, will be vital.

Furthermore, regulatory preparedness for the emergency use of investigational therapeutics must be established to ensure rapid deployment during future resurgences. The 2025–2026 outbreak should serve as a catalyst for strengthening national health security. By sustaining vigilance and enhancing diagnostic and surveillance infrastructure, Ethiopia can ensure that this first encounter with MVD serves as a foundation for a more resilient public health future.

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Human Resources for Health Flooding Strategy on Quality of Antenatal and Intrapartum Care Services in Ethiopia: A Quasi-Experimental Pre–Post Study

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ABSTRACT

Background: Ethiopia has implemented a Human Resources for Health (HRH) flooding strategy since 2011 to rapidly expand the availability of health professionals nationwide. Although this approach is expected to strengthen the healthcare workforce, its effects on healthcare quality and service delivery outcomes have not been comprehensively evaluated.

Objective: This study assessed the association between the HRH flooding strategy and maternal healthcare quality, with a focus on antenatal and intrapartum care, to inform strategies for optimizing the effectiveness of workforce investment.

Methods: A quasi-experimental pre-post design with propensity score matching was employed to evaluate changes in care quality following implementation of the HRH flooding strategy. Data were pooled from two rounds of the Ethiopian Demographic and Health Survey (EDHS), using the 2011 EDHS as the pre-intervention control group and the 2016 EDHS as the post-intervention group. The primary outcomes were the quality of antenatal care (ANC) and intrapartum care provided.

Results: The quality of ANC was 27.1% (95% CI: 25.7–28.6) in the treatment group compared to 8.5% (95% CI: 7.5–9.6) in the control group. Similarly, the quality of intrapartum care was 44.0% (95% CI: 42.4–45.6) and 20.8% (95% CI: 19.3–22.5) in the treatment group and control group, respectively. The average treatment effect of the HRH flooding strategy was associated with a 13.5 percentage-point improvement in the quality of ANC and a 23.3 percentage-point improvement in the quality of intrapartum care. Using a 0.01 caliper, radius matching achieved a strong balance between treatment and control groups.

Conclusion: Implementing the HRH flooding strategy is positively associated with improvements in the quality of ANC and intrapartum care services in Ethiopia. However, these findings should be interpreted with caution, as unmeasured confounding factors, including concurrent health system programs, may have contributed to the observed effect.

Keywords: Human Resources for Health; Maternal Health Services; Quality of Health Care; Propensity Score Matching

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INTRODUCTION

Sustainable Development Goal 3 (SDG 3) aims to reduce the global maternal mortality ratio (MMR) to fewer than 70 per 100,000 live births by 2030, with a strong emphasis on improving maternal health and access to quality healthcare services (1). Despite global progress, Sub-Saharan Africa (SSA) continues to bear a disproportionate burden of maternal and newborn mortality, where the lifetime risk of maternal death remains as high as 1 in 36 women (2). Globally, an estimated 210 million pregnancies occur each year, and many adverse pregnancy outcomes can be prevented or mitigated through timely and appropriate antenatal care (ANC) (3, 4).

In 2016, the World Health Organization (WHO) issued updated, comprehensive ANC guidelines to safeguard the health of pregnant women and their fetuses (5). Although coverage of maternal and child health services expanded during the Millennium Development Goal (MDG) era, accumulating evidence indicates that improvements in service quality have lagged behind gains in access (6, 7).

Healthcare quality refers to the extent to which health services increase the likelihood of desired health outcomes for individuals and populations and is defined as a core component of universal health coverage (8). The National Academy of Medicine defines quality healthcare as timely, efficient, equitable, patient -centered, safe, and effective (9). Achieving meaningful reductions in maternal and perinatal morbidity and mortality in low-income countries, therefore, requires substantial investment not only in expanding access and utilization of maternity services but also in improving the quality of care delivered (10).

Both measuring the quality of maternal healthcare and identifying its determinants are essential for improving current and future health outcomes in low-income countries (11). While considerable attention has been paid to increasing access to healthcare during pregnancy, childbirth, and the postnatal period, far less emphasis has been placed on ensuring that all recommended evidence-based guidelines are consistently upheld during antenatal, intrapartum, and postnatal care (12). Ethiopia, in particular, must accelerate improvements in the quality of maternal healthcare to achieve the SDG targets of reducing maternal mortality to fewer than 70 per 100,000 live births and neonatal mortality to fewer than 12 per 1,000 live births by 2030 (7).

Strengthening human resources for health (HRH) is a critical component of achieving these targets and represents one of the six core building blocks of the WHO health systems framework (13). HRH plays a central role in health system performance and accounts for a substantial proportion of health sector expenditure. Recognizing this, Ethiopia has prioritized HRH development as a cornerstone of its health sector transformation agenda and has implemented several HRH initiatives through successive health sector development plans (14). Nonetheless, persistent challenges remain, including low workforce density, inequitable distribution, and inefficient utilization of available personnel (14-17). In response, the Ethiopian government, in collaboration with the United States Agency for International Development (USAID), launched the Strengthening Human Resources for Health program (2012–2018) to address critical HRH gaps (18, 19).

In 2010, the WHO and the Global Health Workforce Alliance (GHWA) recommended a “flooding strategy” to rapidly increase the health workforce, particularly in countries facing severe shortages. Ethiopia has been classified as experiencing a health workforce crisis, with only 0.3 physicians, nurses, and midwives per 10,000 population—far below the critical threshold of 23 per 10,000 (20).

To address this shortage and align with the WHO recommendation of at least 10 physicians per 100,000 population in low-income countries (21), the Ethiopian Ministries of Health and Education substantially expanded pre-service training capacity. This included establishing new medical schools and health professional training programs, increasing the number of medical schools from three to over 33, and producing more than 3,000 graduates annually. The first large cohort of graduates entered the workforce between 2011 and 2012 (22-25).

Despite this rapid expansion, many training institutions continue to face shortages of qualified faculty, and the health system experiences high physician turnover. The HRH flooding strategy was therefore designed not only to rapidly scale up the health workforce but also to address longstanding geographic inequities, particularly in underserved rural areas (26-28). To advance universal access to primary healthcare, HRH reforms were implemented in phases, beginning with community-level services delivered by health extension workers and gradually expanding to health centers and hospitals (27, 28).

However, critics argue that the flooding approach may prioritize rapid workforce expansion at the expense of other critical of HRH development, such as quality, retention, and performance management dimensions(20). Against this backdrop, the present study aims to assess the impact of the HRH flooding strategy on the quality of antenatal care and intrapartum care services in Ethiopia using a quasi-experimental design.

METHODS

Data source

This study used data from the 2011 and 2016 Ethiopian Demographic and Health Surveys (EDHS), which are nationally representative, population-based cross-sectional surveys conducted as part of a global program funded by the United States Agency for International Development (USAID). The surveys collected comprehensive demographic and health information from women of reproductive age and young children. A multistage stratified cluster sampling design was employed, whereby households were selected within enumeration areas (clusters) (29).

The analysis included a total of 6,228 weighted respondents for the assessment of antenatal care (ANC) quality and 6,058 weighted respondents for intrapartum care quality, all of whom had given birth within the three years preceding each survey. The women individual record files from the 2011 and 2016 EDHS were used. Access to the datasets was granted upon approval from DHS office, obtained through their official website (http://www.measuredhs.com).

Outcome variable

This study assessed two broad outcome variables: the quality of ANC and the quality of intrapartum care.

Quality of antenatal care (ANC): was measured using five essential service components: (1) blood pressure measurement, (2) provision of iron supplementation, (3) blood testing for infection and anemia, (4) urine testing for bacteriuria and proteinuria, and (5) counseling on pregnancy danger signs. These indicators have been widely used in previous studies as proxy measures of ANC quality (30, 31). Women who received all five components were classified as having received quality ANC (coded as "1"), whereas those missing one or more components were classified as not having received quality ANC (coded as "0").

Quality of intrapartum care: was assessed using three indicators: (1) delivery in a health facility, (2) attendance by a skilled birth attendant, and (3) initiation of breastfeeding within one hour of birth. These indicators have been commonly employed to measure intrapartum care quality in prior studies (32, 33). Respondents who received all three components were classified as having received quality intrapartum care (coded as "1"), while those missing any component were classified as not having received quality intrapartum care (coded as "0").

Treatment variable

The treatment variable was the survey year, used as a proxy for exposure to the Human Resources for Health (HRH) flooding strategy. Respondents from the 2011 EDHS, conducted before implementation of the HRH flooding strategy, were designated as the control group, whereas respondents from the 2016 EDHS, conducted after implementation, were classified as the treatment group. This variable was coded as "1" for the treatment group and "0" for the control group.

Several covariates known to influence the quality of maternal healthcare services were considered, including maternal age, place of residence, women's educational status, employment status, partner's employment status, wealth index, marital status, timing of the first ANC visit, number of ANC visits, birth order, and religion. Rao–Scott chi-square tests were conducted using the survey ("svy") command, with v023 specified as the stratification variable and v021 as the primary sampling unit. Covariates with P-values < 0.05 were retained for inclusion in the propensity score matching process.

Data analysis

Radius matching with a caliper of 0.01 was employed to estimate the association between the HRH flooding strategy and the quality of ANC and intrapartum care services. Propensity score matching (PSM) is a widely used quasi-experimental method for estimating causal effects when randomization is not feasible, as it reduces selection bias by balancing observable characteristics between treatment and control groups (34). Propensity scores were estimated using a logit model via the Stata pscore command. Matching was then performed using the psmatch2 command with radius matching and a 0.01 caliper. This approach enabled estimation of the Average Treatment Effect on the Treated (ATT), representing the effect of the HRH flooding strategy on service quality among respondents surveyed after its implementation. The Average Treatment Effect on the Untreated (ATU) was also estimated to

predict the potential effect of the HRH flooding strategy on respondents in the 2011 EDHS had they been exposed to the intervention. Additionally, the Average Treatment Effect (ATE) for the overall population was calculated.

The common support assumption was assessed by visually inspecting histograms and kernel density plots of propensity scores for treatment and control groups to ensure sufficient overlap. Balance diagnostics were conducted using the pstest command, examining mean and median bias, standardized percentage bias, t-statistics, pseudo R^2, and likelihood ratio (LR) chi-square statistics before and after matching to evaluate the quality of the matching process.

Sensitivity analysis

A sensitivity analysis was conducted to assess the robustness of propensity score-matching estimates to potential bias from unobserved confounders. The Mantel–Haenszel test statistic was applied to evaluate the sensitivity of the estimated treatment effects, accounting for the binary nature of the outcome variables (35).

RESULTS

A total of 6,228 weighted respondents who had given birth within the three years preceding the survey were included in the analysis of the first outcome: quality of ANC. Of these, 2,519 respondents were in the control group and 3,709 in the treatment group. The proportion of women who received quality ANC was 27.17% (95% CI: 25.7–28.6) in the treatment group, compared with 8.54% (95% CI: 7.53–9.68) in the control group.

For the second outcome, quality of intrapartum care, 6,058 weighted respondents who had given birth in the past three years were included. The quality of intrapartum care services was 44.0% (95% CI: 42.4–45.6) among the treatment group, whereas only 20.8% (95% CI: 19.3–22.5) of respondents in the control group received quality intrapartum care **(Table 1)**.

Estimation of Propensity Score

Propensity scores were estimated using logistic regression based on selected covariates to balance baseline characteristics between the treatment and control groups. The matching process resulted in eight blocks, with a region of common support ranging from 0.24 to 0.84. The mean propensity score was 0.58 (standard deviation: 0.09), indicating adequate overlap between the two groups.

Impact of HRH on the quality of ANC and intrapartum care

Radius matching with a caliper of 0.01 was applied to estimate the impact of the HRH flooding strategy. In the unmatched sample, respondents in the treatment group had a 13.4 percentage-point higher likelihood of receiving quality ANC and a 23.1 percentage-point higher likelihood of receiving quality intrapartum care compared with those in the control group.

After matching, the estimated Average Treatment Effect on the Treated (ATT) indicated that exposure to the HRH flooding strategy was associated with a 13.5 percentage-point increase in the probability of receiving quality ANC (95% CI: 11.3–15.4). Similarly, the HRH flooding strategy was associated with a 23.3 percentage-point increase in the likelihood of receiving quality intrapartum care (95% CI: 21.4–25.3). These findings suggest that women surveyed after the implementation of the HRH flooding strategy had a significantly higher chances of receiving high-quality antenatal and intrapartum care services compared with those surveyed before the strategy was introduced.

Quality of matching

Common support

The assessment of the common support condition indicated that only a small number of observations fell outside the overlap region between the treatment and control groups. For the first outcome variable (quality of ANC), six observations (five untreated and one treated) were outside the common support region and were excluded from the analysis. For the second outcome variable (quality of intrapartum care), five observations (three untreated and two treated) were similarly excluded (**Table 2**). Visual inspection of the propensity score distributions demonstrated substantial overlap between the treatment and control groups (**Figures 1** and **2**), confirming that the common support assumption for propensity score matching was satisfied.

Table 1: Propensity score matching estimates of average treatment Effect

Variable	Sample	Treated	Control	Difference in Proportions	S. E	T-stat
Quality of ANC care	Unmatched	0.18819	0.0539	0.1342	0.0085	15.74
	ATT	0.18818	0.0528	0.1353	0.0081	16.57
	ATU	0.05292	0.1847	0.1308		
	ATE			0.1327		
Quality of intrapartum care	Unmatched	0.4404	0.2087	0.2316	0.0120	19.30
	ATT	0.4404	0.2065	0.2338	0.0122	19.09
	ATU	0.208	0.4571	0.2489	0.2489	
	ATE			0.2401		

Table 2: Common Support

Treatment assignment (ANC)	Off support	On support	Total
Untreated	1	2,596	2,597
Treated	5	3,656	3,661
Total	**6**	**6,252**	**6,258**
Intrapartum care			
Untreated	3	2,521	2,524
Treated	2	3,558	3,560
Total	**5**	**6,079**	**6,084**

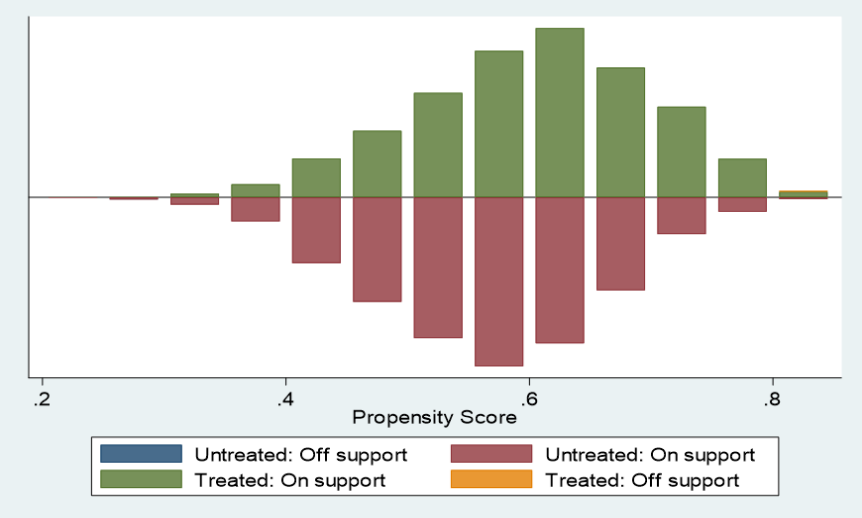

Figure 1: *Propensity score histogram by treatment status for ANC visit*

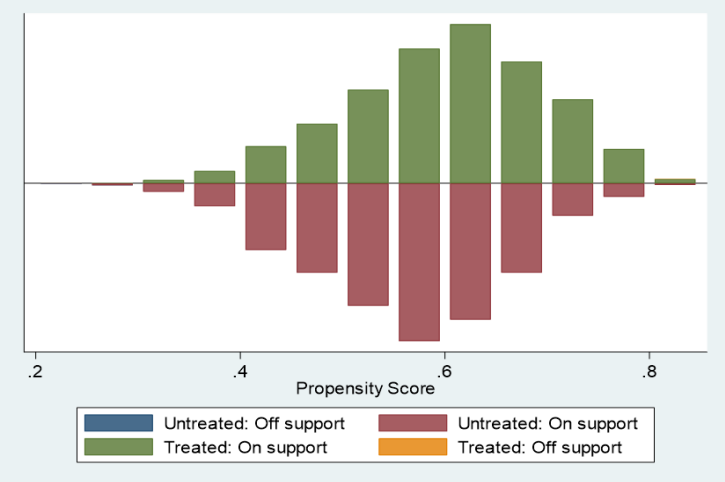

Figure 2: *Propensity score histogram by treatment status for intrapartum visit*

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Balancing test

The matching procedure achieved a good balance across all covariates between the treatment and control groups. After matching, none of the covariates showed statistically significant differences between groups, with all p-values exceeding 0.05. Furthermore, the Pseudo R^2 decreased markedly from 0.029 before matching to 0.000 after matching, and the likelihood ratio (LR) chi-square statistic declined from 246.31 to 3.10, with a corresponding p-value of 0.989.

Substantial improvements were also observed in standardized balance measures. The mean bias was reduced from 9.1 to 1.0, and the median bias declined from 8.9 to 0.7. Similarly, the standardized mean difference (B) decreased from 40.8 to 4.1 (**Table 3**). Consistent with these findings, (**Figure 3)** illustrates that the standardized percentage bias across all covariates was close to zero following matching, further indicating a high-quality match.

Table 3: Balancing Test before and after matching

Variable	Sample	Mean		%bias	%bias reduction	T test	
		Treated	Control			t	p>|t|
Birth order	Unmatched	2.03	2.04	-1.3		-0.52	0.603
	Matched	2.03	2.03	-0.3	75.8	-0.14	0.891
Religion	Unmatched	2.07	2.01	6.8		2.65	0.008
	Matched	2.07	2.07	-0.4	94.2	-0.17	0.867
Timing of ANC	Unmatched	1.61	1.68	-13.3		-5.18	0.000
	Matched	1.611	1.61	-0.2	98.3	-0.10	0.924
Number of ANC visits	Unmatched	1.54	1.46	17.0		6.61	0.000
	Matched	1.54	1.55	-1.3	92.2	-0.57	0.569
Wealth Index	Unmatched	2.02	2.21	-20.4		-7.92	0.000
	Matched	2.02	2.03	-1.0	95.3	-0.40	0.687
Marital Status	Unmatched	1.05	1.07	-8.8		-3.46	0.001
	Matched	1.05	1.05	-0.4	95.5	-0.18	0.854
Maternal Employment Status	Unmatched	0.30	0.34	-8.9		-3.48	0.001
	Matched	0.30	0.32	-1.9	78.6	-0.83	0.408
Partners Education	Unmatched	0.94	0.92	2.5		0.95	0.342
	Matched	0.94	0.94	0.5	80.5	0.20	0.839
Maternal Age	Unmatched	1.87	1.88	-0.9		-0.34	0.732
	Matched	1.87	1.89	-2.5	-182.8	-1.06	0.290
Maternal Education	Unmatched	0.74	0.65	10.4		4.00	0.000
	Matched	0.74	0.75	-0.7	93.0	-0.30	0.764
Residence	Unmatched	1.71	1.67	9.4		3.69	0.000
	Matched	1.71	1.71	1.6	82.6	0.71	0.477
Sample	Ps R2	LR chi2	p>chi2	Mean bias	Med bias	B	R
Unmatched	0.029	246.31	0.000	9.1	8.9	40.8*	1.05
Matched	0.000	3.10	0.989	1.0	0.7	4.1	1.04

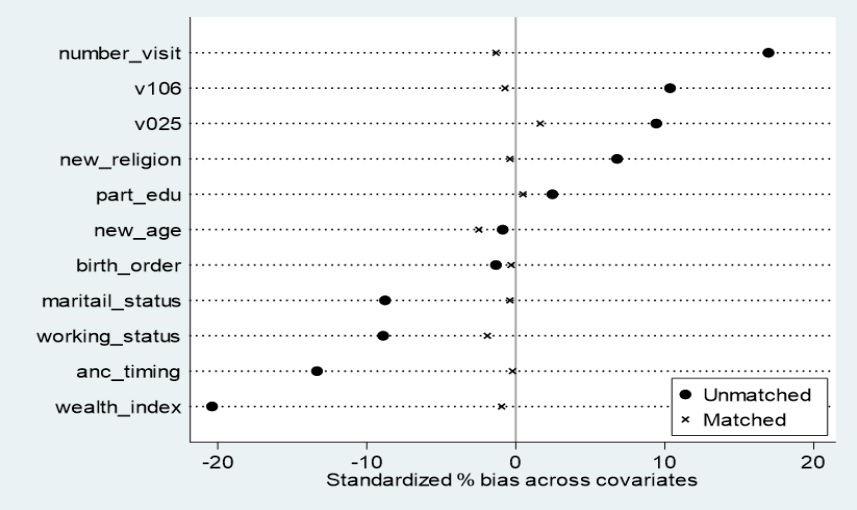

Figure 3: *Standardized % bias across covariates*

Sensitivity analysis

Sensitivity analysis suggested that the estimated treatment effects were robust to potential hidden bias for both outcome variables. The Mantel–Haenszel test statistics (Q_mh+ and Q_mh−) were similar for both outcomes when the sensitivity parameter (Γ) was equal to 1, indicating no hidden bias. As Γ increased to 2, the Mantel–Haenszel test became statistically significant, suggesting that the estimated effects would only be sensitive to relatively strong unobserved confounding **(Table 4 and 5).**

Table 4: Sensitivity analysis for the quality of the ANC service

Gamma	Q_mh+	Q_mh-	P_mh+	P_mh-
1	18.31	18.31	0	0
1.05	17.61	19.02	0	0
1.1	16.95	19.71	0	0
1.15	16.32	20.36	0	0
1.2	15.72	21.00	0	0
1.25	15.14	21.61	0	0
1.3	14.60	22.20	0	0
1.35	14.08	22.78	0	0
1.4	13.58	23.88	0	0
1.45	13.09	23.88	0	0
1.5	12.63	24.41	0	0
1.55	12.19	24.92	0	0
1.6	11.76	25.42	0	0
1.65	11.35	25.91	0	0
1.7	10.95	26.85	0	0
1.75	10.56	26.85	0	0
1.8	10.18	27.31	0	0
1.85	9.82	27.75	0	0
1.9	9.47	28.19	0	0
1.95	9.13	28.62	0	0
2	8.79	29.04	0	0

Gamma: odds of differential assignment due to unobserved factors
Q_mh+: Mantel-Haenszel statistic (assumption: overestimation of treatment effect)
Q_mh-: Mantel-Haenszel statistic (assumption: underestimation of treatment effect)
p_mh+: significance level (assumption: overestimation of treatment effect)
p_mh-: significance level (assumption: underestimation of treatment effect)
Gamma represents the odds of differential assignment to the intervention group due to an unobserved confounder. For example, **Gamma = 2** implies an unobserved factor could double the odds of being in the post-policy (2016) group.

Table 5: Sensitivity analysis for the quality of the Intrapartum care service

Gamma	Q_mh+	Q_mh-	P_mh+	P_mh-
1	18.71	18,71	0	0
1.05	17.85	19.58	0	0
1.1	17.03	20.41	0	0
1.15	16.24	21.21	0	0
1.2	15.50	21.98	0	0
1.25	14.79	22.72	0	0
1.3	14.10	23.43	0	0
1.35	13.45	24.12	0	0
1.4	12.82	24.79	0	0
1.45	12.21	25.43	0	0
1.5	11.63	26.06	0	0
1.55	11.06	26.67	0	0
1.6	10.52	27.26	0	0
1.65	9.99	27.83	0	0
1.7	9.48	28.39	0	0
1.75	8.99	28.94	0	0
1.8	8.51	29.47	0	0
1.85	8.04	29.99	<0.000	0
1.9	7.59	30.50	<0.000	0
1.95	7.14	30.99	<0.000	0
2	6.71	31.48	<0.000	0

Gamma: odds of differential assignment due to unobserved factors

Q_mh+: Mantel-Haenszel statistic (assumption: overestimation of treatment effect)

Q_mh-: Mantel-Haenszel statistic (assumption: underestimation of treatment effect)

p_mh+: significance level (assumption: overestimation of treatment effect)

p_mh-: significance level (assumption: underestimation of treatment effect)

Gamma represents the odds of differential assignment to the intervention group due to an unobserved confounder. For example, **Gamma = 2** implies an unobserved factor could double the odds of being in the post-policy (2016) group.

DISCUSSION

This study demonstrated a substantial improvement in the quality of maternal healthcare services following the implementation of Ethiopia's HRH flooding strategy. The quality of ANC was markedly higher in the treatment group (27.17%; 95% CI: 25.7–28.6) compared with the control group (8.54%; 95% CI: 7.53–9.68). Similarly, the quality of intrapartum care services was considerably higher among women in the treatment group (44.0%; 95% CI: 42.4–45.6) than among those in the control group (20.8%; 95% CI: 19.3 –22.5). These findings suggest that the HRH flooding strategy was positively associated with significant improvements in the quality of both antenatal and intrapartum care services in Ethiopia.

The propensity score–matched analysis further indicated that implementation of the HRH flooding strategy was associated with a 13.5 percentage-point increase in the quality of ANC and a 23.3 percentage-point increase in the quality of intrapartum care services. These improvements underscore the critical role of expanding the health workforce in addressing persistent gaps in maternal healthcare quality.

The observed improvements may be explained by reduced provider-to-patient ratios, greater availability of skilled

health professionals, and enhanced access to care delivered by qualified practitioners, core objectives of the health workforce flooding strategy. This is particularly relevant in Ethiopia, where critical shortages of health personnel have historically constrained progress toward universal health coverage and improved maternal health outcomes (36).

Our findings are consistent with more recent evidence using 2019 data, which reported higher levels of quality ANC (36%) and comparable intrapartum care quality (43%) (33). This alignment suggests that the positive effects of the HRH flooding strategy may have persisted beyond the initial implementation period (2012–2018) (18). The observed improvements may also reflect complementary efforts under the HRH Project, including collaboration with the Higher Education Relevance and Quality Agency (HERQA) to strengthen accreditation, audit, and quality assurance mechanisms in health professional education. These initiatives aimed to enhance training standards, regulatory oversight, and institutional capacity, thereby contributing to improvements in service quality (19).

High-impact maternal health interventions, such as quality antenatal, intrapartum, and postnatal care, are essential for preventing adverse outcomes, including maternal and perinatal morbidity and mortality (37). However, ensuring access to high-quality care remains a major challenge in many low- and middle-income countries (LMICs), where health systems are often fragile and under-resourced (38-40). Ethiopia, in particular, must accelerate progress to meet the Sustainable Development Goals (SDGs), including reducing maternal mortality to fewer than 70 per 100,000 live births, achieving universal skilled birth attendance, and reducing neonatal mortality to fewer than 12 deaths per 1,000 live births by 2030 (41). This finding indicates that the HRH policy is playing a role in achieving the SDGs.

Strengths and Limitations

This study has several strengths. The use of nationally representative DHS data from two time points allowed for a robust comparison of maternal healthcare quality before and after implementation of the HRH flooding strategy. Employing propensity score matching strengthened causal inference by balancing observable characteristics between treatment and control groups, thereby approximating a randomized experimental design. Additionally, the large sample size enhances the generalizability of the findings to the Ethiopian population.

Nevertheless, some limitations should be acknowledged. While propensity score matching adjusts for observed confounders, it cannot account for unmeasured factors that may have influenced both exposure and outcomes. Concurrent health system reforms, local economic changes, or sociocultural shifts between 2011 and 2016 may have affected service quality independently of the HRH flooding strategy. As such, the findings should be interpreted with caution.

Policy Implication

The findings of this study indicate that the HRH flooding strategy was effective in improving the quality of antenatal and intrapartum care services in Ethiopia. Continued investment in the training, recruitment, equitable distribution, and retention of healthcare workers is therefore essential to sustain and further enhance gains in maternal healthcare quality.

Policymakers should consider integrating the HRH flooding strategy with complementary interventions, including strengthening health facility infrastructure, ensuring the availability of essential medical supplies, and addressing financial, geographic, and sociocultural barriers to care. Regular monitoring and evaluation of service quality following HRH interventions will also be critical for identifying implementation gaps and guiding evidence-based policy adjustments.

CONCLUSION

This study provides evidence that Ethiopia's HRH flooding strategy was positively associated with improvements in the quality of antenatal and intrapartum care services. Although unobserved factors and concurrent interventions may have influenced the results, the findings highlight the importance of strategic investments in the health workforce as a pathway to improving maternal health outcomes and advancing progress toward the Sustainable Development Goals.

Abbreviation

PSM: Propensity score matching
DHS: Demographic and Health Survey
HRH: Human Resource for Health
CI: Confidence Interval
ATT: Average Treatment Effect among Treated
ATU: Average Treatment effect on Untreated
ATE: Average Treatment effect on the whole population
ANC: Antenatal Care

Declarations

Availability of data

The datasets used in this study are publicly available from the Measure DHS website: https://dhsprogram.com/data/available-datasets.cfm.

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The authors would like to sincerely acknowledge DHS for approving our acquisition, use, and study of the DHS dataset.

Author's contribution

A.K. conceptualized the study, conducted the literature review, contributed to methodology, performed the analysis, interpreted the results, and prepared the manuscript. E.D., M.J., M.G.T., G.T., and D.M.G. contributed to methodology, formal analysis, and interpretation of the data. K.A.D. contributed to conceptualization, methodology, analysis, and interpretation of the results and revised the manuscript. All authors have read and approved the final version of the manuscript.

Ethical review

This study used pre-existing, publicly available data from www.measuredhs.com, with all identifiers removed; therefore, ethical approval was not required. Permission to access and use the data was obtained from Measure DHS through an online request.

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Clinical trial number

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Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Factor Structure Analysis and Total Score Correlation of the Insomnia Severity Index and the Ford Insomnia Response to Stress Test in Patients with Multiple Somatic Complaints

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ABSTRACT

Background: Insomnia and sleep reactivity are common among individuals presenting with multiple somatic complaints. The Insomnia Severity Index (ISI) and the Ford Insomnia Response to Stress Test (FIRST) are widely used instruments for assessing insomnia severity and vulnerability to stress-related sleep disturbance, respectively. However, their factor structures and interrelationships have not been adequately examined in Nigerian population.

Objective: To determine the factor structure and internal consistency of the ISI and FIRST and to assess the correlation between their total scores among patients with multiple somatic complaints.

Methods: A cross-sectional study was conducted among 111 adult psychiatric outpatients with multiple somatic complaints at the Obafemi Awolowo University Teaching Hospitals Complex, Nigeria. Sociodemographic data were collected, and the participants completed the ISI and FIRST questionnaires. Exploratory factor analysis using maximum likelihood extraction with varimax rotation was applied to the ISI, while principal component analysis was used for the FIRST. Internal consistency was assessed using Cronbach's alpha. Correlation and simple linear regression analyses were performed to evaluate the associations between the instruments.

Results: Data from 110 participants were analyzed (mean age 39.4 ± 8.7 years; 58.2% female). The ISI demonstrated a two-factor structure explaining 47.22% of the variance, with an overall Cronbach's alpha of 0.67. The FIRST demonstrated a single-factor structure explaining 56.40% of the variance, with excellent reliability (Cronbach's alpha = 0.90). A significant positive correlation was observed between ISI and FIRST scores ($r = 0.40$, $p < 0.01$). Linear regression analysis showed that sleep reactivity accounted for 15% of the variance in insomnia severity (Adjusted $R^2 = 0.150$, $p < 0.01$).

Conclusion: The ISI and FIRST demonstrated acceptable construct validity and reliability in this population. Sleep reactivity was significantly associated with insomnia severity, supporting the clinical utility of the FIRST in identifying individuals at risk of insomnia.

***Keywords*:** Insomnia Severity Index, Ford Insomnia Response to Stress Test, insomnia, sleep reactivity, factor analysis, somatic symptoms

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INTRODUCTION

Multiple somatic complaints are frequently encountered in general medical and psychiatric practice and are associated with substantial functional impairment and healthcare utilization. In the ICD-10, they are categorized under somatoform disorders (1), characterized by repeated presentation of physical symptoms and persistent requests for medical investigations, despite negative findings. In the International Classification of Diseases, 11th Revision (ICD-11), these conditions are categorized under bodily distress disorder, characterized by distressing bodily symptoms and excessive attention directed toward them (1, 2). These conditions often co-occur with depression and anxiety (1,2). Insomnia is highly prevalent among patients with psychiatric disorders, including those with multiple somatic complaints. Insomnia contributes to impaired functioning, poor quality of life, and worsening psychiatric symptoms. Reliable and valid tools are essential for accurate assessment of insomnia and related risk factors.

The Insomnia Severity Index (ISI) is a widely used self-report instrument assessing insomnia severity, its impact on daily functioning, and associated distress (3). The Ford Insomnia Response to Stress Test (FIRST) measures sleep reactivity, defined as the predisposition to experience sleep disturbances in response to stress (4).

According to the behavioral model of insomnia (5), insomnia arises from the interaction of trait vulnerabilities and precipitating factors. Individuals with high sleep reactivity (FIRST) may thus be more susceptible to insomnia (ISI), suggesting a potential positive correlation between the two measures. Therefore, a positive association between sleep reactivity and insomnia severity is expected. Despite widespread use of the ISI and FIRST globally, their psychometric properties and interrelationship have not been examined among patients with multiple somatic complaints in the Nigerian setting. This study aimed to (1) determine the factor structures of the ISI and FIRST, and (2) examine the correlation between their total scores in patients with multiple somatic complaints.

MATERIALS AND METHODS

Study setting and design

A cross-sectional study was conducted among psychiatric outpatients at Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.

Participants

Adult patients aged 18–64 years presenting with multiple somatic complaints were consecutively recruited. Patients identified with psychotic disorders, psychoactive substance use disorders, or recent psychotropic medication use (within one month), according to the 10th Edition of the International Classification of Diseases (ICD-10) by the World Health Organization (1) were excluded.

Data Collection

Participants provided sociodemographic information and completed both the Insomnia Severity Index (ISI) and Ford Insomnia Response to Stress Test (FIRST).

Instruments

Insomnia Severity Index (ISI)

The ISI is a 7-item self-report instrument assessing insomnia severity over the past week. Each item is rated on a 0–4 scale, with total scores ranging from 0 to 28. Higher scores indicate greater insomnia severity (3). Scores ≥15 indicate clinically significant insomnia (3). Severity grades of insomnia on the ISI include the following: no clinically significant insomnia (0-7), subthreshold insomnia (8–14), clinical insomnia of moderate severity (15–21), and severe clinical insomnia (22–28) (6, 7, 8). In the present study, subjects who scored ≥15 were considered to have clinically significant insomnia, as has been done in previous studies (6, 7, 8). The ISI has been demonstrated to have an acceptable validity and reliability profile (9).

Ford Insomnia Response to Stress Test (FIRST)

The FIRST is a 9-item self-report questionnaire measuring sleep reactivity to stress (4). It requires the subjects to rate their likelihood of having sleep disruption in a variety of stressful situations during the day or evening. Items are rated on a 4-point Likert scale, with total scores ranging from 9 to 36. Scores ≥18 indicate high sleep reactivity (10). This study used this (≥ 18) as the cut-off score.

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Statistical Analysis

Data was analyzed using the Statistical Package for Social Sciences (SPSS) software, version 23. The general characteristics of the subjects were determined using descriptive statistics.

Exploratory factor analysis was performed to evaluate the construct validity and underlying dimensional structure of the Insomnia Severity Index (ISI) and the Ford Insomnia Response to Stress Test (FIRST). Factor analysis is a multivariate statistical technique used to identify latent constructs that explain correlations among observed variables and to assess whether questionnaire items measure distinct theoretical domains. The suitability of the data for factor analysis was assessed by examining the correlation matrix and sampling adequacy. Factor extraction was conducted using maximum likelihood extraction with varimax rotation for the ISI. Maximum likelihood extraction was selected because it provides statistically robust estimates of factor loadings and allows identification of latent constructs underlying insomnia severity. Varimax rotation was applied to enhance interpretability by maximizing the variance of factor loadings and improving the distinction between factors.

Principal component analysis (PCA) was used to examine the factor structure of the FIRST. PCA is an appropriate method for identifying the dimensional structure and explaining the maximum proportion of variance among items. Factors with eigenvalues greater than one were retained. Factor loadings of 0.30 or greater were considered significant.

Internal consistency reliability of the instruments and their subscales was assessed using Cronbach's alpha coefficient. Cronbach's alpha values of 0.70 or higher were considered acceptable indicators of reliability, while values above 0.90 indicated excellent reliability.

Pearson correlation analysis and simple linear regression were performed to examine the relationship between ISI and FIRST total scores. Statistical significance was set at $p < 0.05$.

Ethical Considerations

Ethical approval was obtained from the institutional review board. Written informed consent was obtained from all participants.

RESULTS

Participants characteristics

A total of 110 participants were included in the analysis, yielding a response rate of 99.1%. The age of the participants ranged from 24 to 52 years, with a mean age of 39.4 ± 8.7 years. Of the participants, 64 (58.2%) were female, and 46 (41.8%) were male. Forty-one participants (37.3%) were in an intimate relationship, while 69 (62.7%) were not.

Based on the Insomnia Severity Index (ISI), 26 participants (23.6%) had clinical insomnia (ISI score ≥15). In addition, 51 participants (46.4%) demonstrated significant sleep reactivity, as indicated by a Ford Insomnia Response to Stress Test (FIRST) score ≥18.

The descriptive statistics for participants' age and scores on the ISI and FIRST are presented in Table 1, while the clinical diagnoses of the participants are summarized in Table 2.

Table 1: Descriptive statistics of participants' age and scores on the Insomnia Severity Index (ISI) and Ford Insomnia Response to Stress Test (FIRST)

Variable	Range	Mean	Standard Deviation (SD)	Standard Error of Mean (SEM)
Age (years)	24-52	35.9	8.7	0.8
Insomnia Severity Index Score	14-27	20.8	2.9	0.3
Ford Insomnia Response to Stress Score	10-27	19.4	4.0	0.4

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Table 2: Clinical diagnoses of participants according to ICD-10 classification

ICD-10 Diagnosis	Frequency (n)	Percentage (%)
Depressive Episode	49	44.5
Generalized Anxiety Disorder	13	11.8
Phobic Anxiety Disorder	12	11.0
Posttraumatic Stress Disorder	3	2.7
Somatization Disorder	15	13.6
Undifferentiated Somatoform Disorder	18	16.4

Factor structure and reliability of the Insomnia Severity Index (ISI) and Ford Insomnia Response to Stress Test (FIRST)

Exploratory factor analysis of the Insomnia Severity Index (ISI), consisting of seven items, identified a two-factor structure using maximum likelihood extraction with varimax rotation. The two factors collectively accounted for 47.22% of the total variance. The factor loadings and structure are presented in Table 3.

The first factor comprised items related to sleep initiation, sleep maintenance, and early morning awakening, representing the core symptoms of insomnia. The second factor included items related to sleep satisfaction, noticeability of sleep problems, distress or worry about sleep difficulties, and interference with daytime functioning, reflecting the functional and psychological impact of insomnia.

Reliability analysis demonstrated acceptable internal consistency for the overall ISI, with a Cronbach's alpha coefficient of 0.67. The first factor demonstrated a Cronbach's alpha of 0.65, while the second factor demonstrated a Cronbach's alpha of 0.70, indicating acceptable reliability of both subscales.

For the Ford Insomnia Response to Stress Test (FIRST), factor analysis using principal component analysis identified a single-factor structure. This factor accounted for 56.40% of the total variance. All nine items loaded significantly on this factor, indicating that the instrument measures a single underlying construct, sleep reactivity. The factor loadings are presented in Table 4.

The FIRST demonstrated excellent internal consistency reliability, with a Cronbach's alpha coefficient of 0.90, indicating a high level of internal consistency among the items.

Overall, these findings support the construct validity and reliability of both the ISI and FIRST in assessing insomnia severity and sleep reactivity among patients with multiple somatic complaints.

Table 3: Factor Loadings of the Insomnia Severity Index (ISI)

Item Number	Item Description	Factor 1(Nocturnal Sleep Disturbance)	Factor 2 (Perceived Impact of Sleep Problems)
1	Severity of early insomnia	0.999 *	-0.001
2	Severity of middle insomnia	0.464 *	0.117
3	Severity of terminal insomnia	0.583 *	0.011
4	Sleep satisfaction	0.273	0.280 *
5	Noticeability of sleep problems	0.032	0.297 *
6	Worry/Distress about sleep difficulties	0.097	0.932 *
7	Interference with daytime functioning	0.219	0.754 *
	Total variance explained: 47.22%		
	Cronbach's alpha: Overall scale: 0.67	0.65	0.70

Extraction method: Maximum likelihood.

Highest loading for each item is marked with an asterisk ().*

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Table 4: Factor Loadings of the Ford Insomnia Response to Stress Test (FIRST)

Item Number	Item Description	Factor Loading
1	Before an important meeting the next day	0.873
2	After a stressful experience during the day	0.715
3	After a stressful experience in the evening	0.766
4	After getting bad news during the day	0.666
5	After watching a frightening movie or TV show	0.941
6	After having a bad day at work	0.719
7	After an argument	0.603
8	Before having to speak in public	0.679
9	Before going on vacation, the next day	0.739

Extraction method: Principal Component Analysis

Total variance explained: 56.40%

Cronbach's alpha: 0.90

Correlation and Regression

Simple linear regression analysis demonstrated a significant positive association between the total scores of the Insomnia Severity Index (ISI) and the Ford Insomnia Response to Stress Test (FIRST) ($r = 0.40$, $p < 0.01$). This indicates that higher sleep reactivity, as measured by the FIRST, significantly predicts greater insomnia severity. The linear relationship accounted for 15% of the variance in ISI scores (Adjusted $R^2 = 0.150$, $p < 0.01$), as summarized in Table 5.

Table 5: Simple Linear Regression Analysis of ISI Scores Predicted by FIRST Scores

Variable	β (SE)	95% Confidence Interval	t	df	p-value
FIRST total score	0.284 (0.063)	0.160 to 0.408	4.536	108	<0.001

Model summary: $r = 0.400$, $R^2 = 0.160$, Adjusted $R^2 = 0.150$, $F(1,108) = 20.57$, $p < 0.001$.

Notes: ISI total score was the dependent variable, and FIRST total score was the independent variable. β = unstandardized regression coefficient; SE = standard error.

DISCUSSION

This study is the first to examine the factor structures of the Insomnia Severity Index (ISI) and the Ford Insomnia Response to Stress Test (FIRST) among patients with bodily distress disorder in this population. It is also the first to demonstrate a significant positive correlation between the total scores of the two instruments.

The ISI demonstrated a two-factor structure, comprising nocturnal sleep disturbances (early, middle, and terminal insomnia) and the perceived impact of sleep problems (sleep satisfaction, noticeability of sleep difficulties, distress/worry, and interference with daytime functioning). Previous studies have reported varying factor solutions for the ISI, including one-, two-, and three-factor models (11). The two-factor solution identified in this study is consistent with prior research, supporting the construct validity of the ISI in patients with multiple somatic complaints. The FIRST demonstrated a single-factor structure, confirming its utility as a unidimensional measure of sleep reactivity, in line with previous studies (12).

Sleep reactivity reflects an individual's trait-like predisposition to stress-related sleep disturbances and is a recognized risk factor for the development of insomnia (13). In this study, 23.6% of participants met the criteria for clinical insomnia (ISI ≥15), while 46.4% exhibited significant sleep reactivity (FIRST ≥18). This suggests that a substan-

tial proportion of individuals with high sleep reactivity do not currently experience insomnia but may be at elevated risk of developing it in the future. Prior research has shown that individuals with high sleep reactivity have more than three times the odds of developing insomnia within one year compared to those with low reactivity (13). Therefore, assessing sleep reactivity using the FIRST can aid in early identification of at-risk individuals, allowing for preventive interventions and targeted management strategies. This has important implications for clinicians, researchers, and patients in the field of sleep medicine.

The significant positive correlation between FIRST and ISI scores ($r = 0.40$, $p < 0.01$) further supports the theoretical model linking trait vulnerability (sleep reactivity) to insomnia severity. Regression analysis showed that sleep reactivity accounted for 15% of the variability in insomnia severity, highlighting its role as a meaningful predictor, though other factors likely contribute to insomnia in this population.

Strengths and Limitations

This study is strengthened by the use of validated instruments, a high response rate (99.1%), and the application of rigorous factor analytic methods. However, the sample size was relatively modest, limiting the generalizability of the findings. Additionally, the cross-sectional design precludes causal inference, and the study was conducted in a single clinical setting, which may not fully represent the broader population. Future studies with larger, multi-center samples and longitudinal designs are warranted to confirm these findings and examine the predictive validity of sleep reactivity over time.

CONCLUSION

The Insomnia Severity Index (ISI) and Ford Insomnia Response to Stress Test (FIRST) demonstrated acceptable construct validity and reliability in patients with multiple somatic complaints. The ISI displayed a two-factor structure, while the FIRST was unidimensional. Sleep reactivity, as measured by the FIRST, was significantly associated with insomnia severity, supporting its utility as a predictive tool. These findings highlight the clinical relevance of assessing both insomnia severity and sleep reactivity to guide early interventions and preventive strategies in high-risk populations.

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Author contributions: CM: Conceptualization, Data collection**,** Analysis, Manuscript writing, and Review and approval.

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Seroprevalence of Hepatitis B Virus Infection among people Living with HIV Attending at the Dangila Health Center, Northwest Ethiopia

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ABSTRACT

Background: Hepatitis B virus (HBV) co-infection remains a significant cause of liver-related morbidity and mortality among people living with HIV/AIDS. Shared transmission routes increase the risk of co-infection, and HIV accelerates the progression of HBV-related liver disease despite widespread antiretroviral therapy (ART). However, there is limited up-to-date evidence on the seroprevalence and associated factors of HBV among HIV patients in the study area.

Objective: To assess the seroprevalence of HBV infection and identify associated risk factors among adults living with HIV attending at the Dangila Health Center, Northwest Ethiopia.

Methods: An institution-based cross-sectional study was conducted from June to August 2020 among 384 HIV-positive adults. Socio-demographic, behavioral, and clinical data were collected using a structured questionnaire and medical record review. Serum samples were tested for hepatitis B surface antigen (HBsAg) and anti-HCV antibodies using rapid test kits. Logistic regression analyses were performed to identify factors independently associated with HBV infection. A p-value <0.05 was considered statistically significant.

Results: The prevalence of HBV infection was 4.4% (17/384), indicating intermediate endemicity. No HCV infection was detected. In multivariate analysis, history of tooth extraction (AOR=3.17; 95% CI: 1.03–9.82), sexually transmitted diseases (AOR=3.53; 95% CI: 1.09–11.47), and multiple sexual partners (AOR=9.68; 95% CI: 2.45–38.24) were independently associated with HBV infection.

Conclusion: HBV co-infection remains a public health concern among HIV-positive individuals in this setting. Routine HBV screening, vaccination, and targeted behavioral interventions should be strengthened within HIV care programs.

Keywords: Hepatitis B, HIV, Co-infection, Risk factors, Ethiopia

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INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic viruses that infect liver cells and cause hepatitis, an inflammatory condition of the liver (1). The clinical spectrum of these infections ranges from asymptomatic carriage and acute self-limiting disease to fulminant hepatitis and chronic liver disease. Chronic HBV and HCV infections are major global public health problems due to their strong association with liver cirrhosis and hepatocellular carcinoma (HCC) (2). Globally, over 2 billion people have been infected with HBV, with approximately 360 million living with chronic infection and at increased risk of cirrhosis and HCC (3). In 2019, HBV caused an estimated 820,000 deaths (4). The prevalence of chronic HBV infection varies geographically, with high endemicity (>8%) in Africa, Asia, and the Western Pacific; intermediate prevalence (2–7%) in Southern and Eastern Europe; and low prevalence (<2%) in North America, Western Europe, and Australia (5).

HBV and HCV share common transmission routes, including exposure to infected blood, sexual contact, and use of contaminated instruments, which increases the likelihood of co-infection or superinfection (6). Individuals co-infected with HBV and HCV usually experience more severe liver disease and have a higher risk of HCC (7). Among people living with HIV/AIDS (PLWHA), HBV infection is a leading cause of non-AIDS-related morbidity and mortality (8).

In Ethiopia, hospital-based studies have reported that HBsAg prevalence among HIV-positive individuals ranges from 3.9% to 14% (9), while HIV/HCV co-infection rates in major urban centers vary from 1.3% to 18.9% (10). Despite the burden of HBV and HCV among PLWHA, routine HBV screening is not consistently implemented in many primary health-care settings, even though it is recommended in the national HIV care guidelines. Moreover, there is limited evidence on the prevalence and risk factors of HBV and HCV infections among HIV -positive individuals in this setting.

Therefore, this study aimed to determine the sero-prevalence of hepatitis B surface antigen (HBsAg) and anti-HCV antibodies and to identify risk factors associated with HBV and HCV infections among people living with HIV/AIDS attending Dangila Health Center, Awi Zone, Northwest Ethiopia.

MATERIAL AND METHODS

Study area

This study was conducted at Dangila Health Center. Dangila is a district in the Awi Zone of the Amhara National Regional State, located in northwestern Ethiopia. It is the largest of three towns within the Dangila District, with an estimated population of 53,225 in 2021 (11). The town's health care delivery system represents the first level of the district health system and includes one primary hospital (serving 60,000–100,000 people, including rural areas), one health center (serving 15,000–25,000 people), and ten satellite health posts (each serving 3,000–5,000 people).

Study design and period

A health institution-based cross-sectional study was conducted from June 2020 to August 2020 to determine the sero-prevalence of HBV and HCV infections and associated risk factors among HIV-positive individuals attending the ART clinic at Dangila Health Center, northwest Ethiopia.

Source and study population

All HIV-positive individuals who visited the ART clinic at Dangila Health Center during the study period constituted the source population. HIV-positive adults who met the inclusion criteria and provided informed consent were the study population.

Inclusion and exclusion criteria

Adults with HIV/AIDS aged ≥18 years and willing to provide a blood sample, were included. Individuals younger than 18 years or those who were severely ill were excluded.

Study variables

The independent variables included socio-demographic factors (age, sex, marital status, occupation, educational status, and income) and potential risk factors (history of unsafe injections, multiple sexual partners, blood transfusion, surgical procedures, ear piercing, tattooing, tooth extraction, sexually transmitted diseases, history of HBV vaccination, and liver disease). The dependent variables were the HBV and HCV infections.

Sample size and sampling technique

The sample size was determined using a single population proportion formula. As the prevalence of HBV and HCV in the study area was unknown, a prevalence of 50% was as-

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sumed. At a 95% confidence interval (Z = 1.96) and a 5% margin of error (d = 0.05), the calculated sample size was 384. After adding 10% to account for potential non-response, the final sample size was 422 participants.

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where:

□ = required sample size

□ = critical value at 5% level of significance (1.96)

□ = expected prevalence of HBV or HCV infection (assumed 50% due to lack of prior data)

□ = margin of error (0.05)

Data collection procedure

Questionnaire survey

Data on socio-demographic characteristics, lifestyle factors, sexual behavior, and potential clinical risk factors were collected using a pre-tested structured questionnaire. The questionnaire was administered by trained nurses working at Dangila Health Center. To ensure clarity and consistency, the tool was pretested on 5% of the sample (n = 21) among HIV-positive individuals who were not included in the main study.

Anthropometric measurements, ART status, and WHO clinical staging

HIV-related clinical data, including World Health Organization (WHO) clinical staging, CD4 T-cell counts, and antiretroviral therapy (ART) status, were retrieved from patients' medical records by trained nurses.

Anthropometric measurements were conducted following standard procedures. Body weight was measured using a calibrated beam balance to the nearest 0.1 kg, with participants wearing light clothing. Height was measured to the nearest 0.1 cm using a standard measuring scale, with participants standing erect, feet together, knees straight, and looking horizontally, without shoes. Both weight and height measurements were taken twice, and the mean values were used for analysis. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Standard adult BMI categories were applied: underweight (<18.5), normal weight (18.5–24.9), overweight (25.0–29.9), and obese (≥30.0).

Blood sample collection, serum separation, and storage

Three milliliters of venous blood were collected from the median cubital vein of each participant into a plain tube by a trained laboratory technologist. To prevent duplicate sampling, a mark was placed on each participant's follow-up card. Blood samples were allowed to clot at room temperature, and serum was separated by centrifugation. The serum was transferred to labeled Nunc tubes and transported to the Microbiology and Biomedical Sciences Laboratory, Biology Department, Bahir Dar University, in an icebox maintained at 2–8°C. Samples were tested within 48–72 hours. Standard procedures were strictly followed for collection, storage, and analysis.

Serological detection of hepatitis B surface antigen (HBsAg)

Serum samples were brought to room temperature prior to testing. HBsAg was qualitatively detected using the HBsAg Rapid Test Kit (CTK Biotech, Inc., San Diego, USA) with a reported sensitivity and specificity of 100%. The test is a lateral flow chromatographic immunoassay. The test cassette contains a conjugate pad with mouse anti-HBsAg antibody conjugated to colloidal gold and a nitrocellulose membrane with a test line (T) and control line (C).

When serum is added to the test device, any HBsAg present binds to the labeled antibody conjugates and subsequently captured at the test (T) line, forming a burgundy-colored line that indicates a positive result. The absence of the colored line at the T-line indicates a negative result. The control (C) line serves as an internal control; if the C-line does not develop, the test is invalid and must be repeated.

Serological detection of anti-hepatitis C antibody (anti-HCV)

Anti-HCV antibodies were detected using the HCV-Ab Plus Rapid Test Cassette (CTK Biotech, Inc., San Diego, USA), with a sensitivity of 98.7% and specificity of 99.6%. This is a double antigen lateral flow immunoassay. The conjugate pad contains recombinant HCV fusion antigen (core, NS3, NS4, NS5) conjugated with colloidal gold, and the membrane strip has a test line (T) coated with the same antigen and a control line (C).

When serum is applied, any anti-HCV antibody present binds to the conjugated antigens and are captured at the T-line, forming a burgundy-colored line indicates a positive result. Absence of the T-line indicates a negative result. The C-line serves as an internal control; tests with an absent C-line are considered invalid and must be repeated.

Quality control

To ensure reliable and accurate data, the questionnaire and laboratory materials were pre-tested before the data collection period. Interviews were conducted by trained personnel. Standard operating procedures (SOPs) were strictly followed for specimen collection and processing. The principal investigator supervised the data collection regularly, and 10% of samples were randomly selected and rechecked blindly to maintain quality control.

Data analysis and interpretation

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics were used to summarize the socio-demographic and clinical characteristics of study participants and the prevalence of HBsAg and anti-HCV antibodies. Associations between HBV infection and socio-demographic or clinical variables were initially assessed using the chi-square test.

Univariate logistic regression analysis was performed to estimate crude odds ratios (COR) for each potential risk factor. Variables with p-values <0.25 in univariate analysis were included in a multivariate logistic regression model to identify independent predictors of HBV infection. A p-value <0.05 was considered statistically significant in all analyses.

Ethical considerations

The study was approved by the Ethical Clearance Committee of the College of Science, Bahir Dar University (RCS/869/2020). A support letter was obtained for Dangila Health Center prior to data collection. Study participants were informed about the purpose and procedures of the study, and written informed consent was obtained before collecting blood samples. Personal information and laboratory results were kept confidential by assigning unique codes to each participant. Participation was voluntary, and participants were free to withdraw from the study at any time without any consequences.

RESULTS

Scio-demographic characteristics and Seroprevalence of HBV and HCV

Of the 422 HIV/AIDS patients initially recruited, 384 of them participated in the study, with a response rate of 91.0%. Majority of the participants, 246 (64.1%) were females. Participants' ages ranged from 18 to 73 years, with a median age of 35 years and a mean (±SD) of 35.9 ± 10.2 years. The largest age group was 31–40 years, comprising 152 participants (39.6%). Regarding marital status, 172 participants (44.8%) were married. About one-third of the participants were privately employed (147, 38.3%) and illiterate (122, 31.8%). Majority of the participants had a monthly income of less than 500 Ethiopian Birr (248, 64.6%). Among the 384 study participants, 17 individuals (4.4%) tested positive for hepatitis B surface antigen (HBsAg). All participants (100%) tested negative for hepatitis C virus (HCV) antibodies (**Table 1**).

Clinical, behavioral risk factors and seroprevalence of HBV

A total of 384 adults living with HIV/AIDS were screened to determine the seroprevalence of hepatitis B surface antigen (HBsAg) and its association with clinical and behavioral risk factors. Of the participants, 17 (4.4%) were positive for HBsAg, indicating HBV and HIV co-infection. The HBV and HIV co-infection was observed across all CD4 T-cell count categories, except those with severe immunosuppression (CD4 <200 cells/µL). The highest prevalence was observed in participants with CD4 counts of 200–349 cells/µL (9.84%), followed by those with ≥500 cells/µL (4.79%). Among ART-naïve individuals, 5.26% were HBsAg-positive compared to 4.28% of those on ART. A gradual increase in the HBsAg sero-positivity was observed in study participants with advancing WHO clinical stage: Stage I (3.94%), Stage II (5.33%), Stage III (5.88%), and Stage IV (7.69%). Co-infection rates were similar across BMI categories: underweight (4.55%), normal weight (4.59%), and overweight (2.94%) (**Table 2**).

Univariate and multivariate logistic regression analysis

In the univariate analysis, sex, educational status, monthly income, and occupational categories showed no statistical significance association with HBV infections ($p > 0.05$). Several behavioral and clinical factors showed significant associated with HBV infection in univariate analysis. Study participants with history of tattooing had approximately three times higher odds HBV infections (COR = 3.08; 95% CI: 1.13–8.38; p = 0.03). A history of tooth extraction was associated with nearly fourfold increased odds (COR = 3.97; 95% CI: 1.45–10.88; p = 0.01), Participants reporting a history of sexually transmitted diseases had about three times greater odds of infection (COR = 3.22; 95% CI: 1.08–9.61; p = 0.04). History of multiple sexual partners had more than sixfold increased odds (COR = 6.34; 95% CI: 1.87–21.50; p = 0.01).

Additionally, history of hospital admission was associated with approximately threefold higher odds of infection (COR = 3.09; 95% CI: 1.10–8.71; p = 0.03). In the final multivariate model, three of the factors remained independently associated with HBV infection (p < 0.05). Adults with a history of tooth extraction had over three times higher odds of HBV infection compared to those without such history (AOR = 3.17; 95% CI: 1.03–9.82; p = 0.04). Those with a history of STDs were 3.5 times more likely to be HBV-positive (AOR = 3.53; 95% CI: 1.09–11.47; p = 0.03). Notably, participants reporting multiple sexual exposures had nearly tenfold higher odds of HBV infection (AOR = 9.68; 95% CI: 2.45–38.24; p = 0.01) compared to those without such exposure **(Table 3).**

Table 1: Frequency distribution of socio-demographic variables and Sero-prevalence of HBV among HIV positive individuals attending Dangila Health Center from June 2020 to August 2020

Characteristics	Category	Frequency n (%)	Sero-prevalence of HBsAg	
			Positive n (%)	Negative n (%)
Sex	Male	138 (35.90)	8 (5.80)	130 (94.20)
	Female	246 (64.10)	9 (3.66)	237 (96.34)
Age category (Years)	18-30	77 (20.10)	2 (2.60)	75 (97.40)
	31-40	152 (39.60)	6 (3.95)	146 (96.05)
	41-50	97 (25.30)	3 (3.09)	94 (96.91)
	51-60	50 (13.00)	4 (8.00)	46 (92.00)
	>60	8 (2.10)	2 (25.00)	6 (75.00)
Marital status	Single	94 (24.50)	4 (4.26)	90 (95.74)
	Divorced	71(18.50)	3 (4.23)	68 (95.77)
	Widowed	47(12.20)	5 (10.64)	42 (89.36)
	Married	172 (44.80)	5 (2.91)	167 (97.09)
Occupational status	Housewives	102 (26.60)	8 (7.84)	94 (92.16)
	Private employed	147(38.30)	1(0.68)	146 (99.32)
	Self-employed	78(20.30)	5 (6.41)	73 (93.59)
	Govt. employed	57 (14.80)	3 (5.26)	54 (94.76)
Educational status	Illiterate	122 (31.80)	5 (4.10)	117 (95.90)
	Primary education	114 (29.70)	4 (3.51)	110 (96.49)
	Secondary education	102 (26.60)	5 (4.90)	97 (95.10)
	College education	46 (12.00)	3(6.52)	43 (93.48)
Monthly income in Ethiopian Birr (ETB)	< 500 ETB	248 (64.60)	11(4.44)	237 (95.55)
	500-1000 ETB	79 (20.60)	2 (2.53)	77 (77.47)
	>1000 ETB	57 (14.80)	4 (7.02)	53 (92.98)
Religion	Christian	344 (89.60)	13 (3.78)	331 (96.22)
	Muslim	40 (10.40)	4 (10.00)	36 (90.00)

Table 2: Frequency distribution of clinical data and potential risk factors of HBV among HIV positive individuals attending Dangila Health Center from June 2020 to August 2020

Variable	Categories	Total n (%)	Sero-prevalence of HBsAg	
			Positive n (%)	Negative n (%)
CD_4 T cell count/µl of blood	< 200	46 (12.00)	0 (0)	46 (100)
	200-349	61(15.90)	6 (9.84)	55 (90.16)
	350-499	110 (28.60)	3 (2.72)	107 (97.28)
	≥500	167 (43.50)	8 (4.79)	159 (95.21)
ART status	ART naïve	57 (14.80)	3 (5.26)	54 (94.74)
	On ART	327 (85.20)	14 (4.28)	313 (95.72)
HIV/AIDS stage	I	279 (72.70)	11(3.94)	268 (96.06)
	II	75 (19.50)	4 (5.33)	71 (94.66)
	III	17 (4.40)	1 (5.88)	16 (94.22)
	IV	13 (3.40)	1 (7.69)	12 (92.31)
BMI (kg/m^2)	< 18.5	132 (34.40)	6 (4.55)	126 (95.44)
	18.5-24.9	218 (56.80)	10(4.59)	208 (95.41)
	25-29.9	34 (8.80)	1 (2.94)	33 (97.06)
Unsafe injection	Yes	14 (3.60))	1(7.10)	13 (92.90%)
	No	370 (96.40)	16 (4.30)	354 (95.70)
Multiple sexual practice	Yes	21(5.50)	4 (19.00%)	17 (81.00%)
	No	363 (94.50)	13 (3.60%)	350 (96.40%)
Having pierced ears	Yes	38 (9.90)	4 (10.50%)	34 (89.50%)
	No	346 (90.10)	13 (3.80%)	333 (96.20%)
Tattoo on body	Yes	75 (19.50)	7 (9.30%)	68 (90.70%)
	No	309 (80.50)	10 (3.20%)	299 (96.80%)
Blood transfusion	Yes	23 (6.00)	1 (4.30%)	22 (95.70)
	No	361 (94.00)	16 (4.40%)	345 (95.60)
Tooth extraction	Yes	62 (16.10)	7(11.30%)	55(88.70%)
	No	322 (83.90)	10(3.10%)	312(96.90)
General surgery	Yes	17 (4.40)	2 (11.80%)	15(88.20)
	No	367 (95.60)	15 (4.10%)	352(95.90)
Liver disease	Yes	18 (4.70)	1 (5.60%)	17 (94.40)
	No	366 (95.30)	16 (4.40%)	350 (95.60)
HBV vaccination	Yes	0 (0.00%)	0 (0.00%)	0 (0.00%)
	No	384 (100)	17 (4.40)	367 (95.60)
STD	Yes	47 (12.20)	5 (10.60%)	42 (89.40%)
	No	337 (87.80)	12(3.60%)	325 (96.40%)
Sex without condom	Yes	13(3.40)	1 (7.70%)	12 (92.30%)
	No	371(96.60)	16 (4.30%)	355 (95.70%)
Hospital admission	Yes	61 (15.90)	6 (9.80%)	55 (90.20%)
	No	323 (84.10)	11(3.40%)	312 (96.60%)
Sharing needles	Yes	8 (2.10)	1 (12.50%)	7 (87.50%)
	No	376 (97.90)	16 (4.30%)	360 (95.70%)

Table 3: Univariate and multivariate logistic analysis of risk variables for sero-prevalence of HBsAg among HIV positive individuals attending Dangila Health Center from June to August 2020

Variable	Category	Prevalence of HBsAg		COR (95% CI; P-value)	AOR (95% CI; P-value)
		Positive N(%)	Negative N(%)		
Sex	Male	8 (5.80)	130 (94.20)	1.62 (0.61-4.30; 0.32)	
	Female	9 (3.66)	237 (96.34)	1:00	
Age (year)	18-30	2 (2.60)	75 (97.40)	1:00	
	31-40	6 (3.95)	146 (96.05)	0.65 (0.13-3.29; 0.60)	0.93(0.17-5.18; 0.93)
	41-50	3 (3.09)	94 (96.91)	0.84 (0.14-5.13; 0.85)	1.20(0.18-8.29; 0.85)
	51-60	4 (8.00)	46 (92.00)	0.31 (0.05-1.74; 0.18)	0.46(0.07-3.00; 0.41)
	>60	2 (25.00)	6 (75.00)	0.08 (0.01-0.67; 0.02)	0.18(0.02-1.89; 0.15)
Marital status	Single	4 (4.26)	90 (95.74)	0.67 (0.18-2.57; 0.56)	0.47(0.12-1.93; 0.30)
	Divorced	3 (4.23)	68 (95.77)	0.68 (0.16-2.92; 0.60)	0.75(0.16-3.45; 0.71)
	Widowed	5 (10.64)	42 (89.36)	0.25 (0.07-0.91; 0.04)	0.37(0.09-1.43; 0.15)
	Married	5 (2.91)	167 -(97.09)	1:00	
Occupational status	Housewives	8 (7.84)	94 (92.16)	0.65 (0.17-2.57; 0.54)	0.76(0.18-3.21;0.71)
	Privately employed	1 (0.68)	146 (99.32)	8.11 (0.83-79.67; 0.07)	7.92(0.78-80.99; 0.08)
	Self employed	5 (6.41)	73 (93.59)	0.81 (0.19-3.54; 0.78)	0.93(0.20-4.30; 0.92
	Govt. employed	3 (5.26)	54 (94.76)	1:00	
Educational status	Illiterate	5 (4.10)	117 (95.90)	1.63 (0.37-7.13; 0.51)	
	Primary education	4 (3.51)	110 (96.49)	1.92 (0.41-8.91; 0.41)	
	Secondary education	5 (4.90)	97 (95.10)	1.35(0.31-5.92; 0.69)	
	College education	3 (6.52)	43 (93.48)	1:00	
Monthly income in Ethiopian Birr (ETB)	< 500 ETB	11 (4.44)	237 (95.55)	1.63(0.50-5.31; 0.42)	
	500-1000 ETB	2 (2.53)	77 (77.47)	2.91(0.51-16.44; 0.23)	
	>1000 ETB	4 (7.02)	53 (92.98)	1:00	
Religion	Christian	13 (3.78)	331 (96.22)	2.83 (0.88-9.14; 0.82)	
	Muslim	4 (10.00)	36 (90.00)	1:00	
Unsafe injection	Yes	1 (7.10)	13 (92.90)	1.70 (0.21- 13.82; 0.62)	
	No	16 (4.30)	354 (95.70)	1:00	
Multiple sexual practice	Yes	4 (19.00)	17 (81.00)	6.34(1.87-21.50; 0.01)	9.68(2.45-38.24; 0.01*)
	No	13 (3.60)	350 (96.40)	1:00	1:00
Having pierced ears	Yes	4 (10.50)	34 (89.50)	3.01(0.93-9.76; 0.07)	3.09(0.80-11.98; 0.10)
	No	13 (3.80)	333 (96.20)	1:00	1:00
Tattoo on body	Yes	7 (9.30)	68 (90.70)	3.08 (1.13-8.38; 0.03)	2.59(0.83-8.08;0.09)
	No	10 (3.20)	299 (96.80)	1:00	1:00
Blood transfusion	Yes	1 (4.30)	22 (95.70)	0.98 (0.12-7.73;0.98)	
	No	16 (4 .40)	345 (95.60)	1:00	

*Key: COR (Crude odd ratio), AOR (Adjusted odd ratio), *P value <0.05 significant, 1:00 = reference value*

Cont'd…….

Variable	Category	Prevalence of HBsAg		COR (95% CI; P-value)	AOR (95% CI; P-value)
		Positive N(%)	Negative N(%)		
Tooth extraction	Yes	7 (11.30)	55 (88.70)	3.97 (1.45-10.88; 0.01)	3.17(1.03-9.82; 0.04*)
	No	10 (3.10)	312 (96.90)	1:00	1:00
General surgery	Yes	2 (11.80)	15 (88.20)	3.13 (0.66-14.94 ; 0.13)	1.85(0.27-12.57; 0.67)
	No	15 (4.10)	352 (95.90)	1:00	1:00
Liver disease	Yes	1 (5.60)	17 (94.40)	1.29 (0.16-10.28;0.81)	
	No	16 (4.40)	350 (95.60)	1:00	
HBV vaccination	Yes	0 (0.00)	0 (0.00)	NA	
	No	17 (4.40)	367 (95.60)		
STDs	Yes	5 (10.60)	42 (89.40)	3.22 (1.08-9.61; 0.04)	3.53(1.09-11.47; 0.03*)
	No	12 (3.60)	325 (96.40)	1:00	1:00
Sex without condom	Yes	1 (7.70)	12 (92.30)	1.85 (0.23-15.11;0.57)	
	No	16 (4.30)	355 (95.70)	1:00	
Hospital admission	Yes	6 (9.80)	55 (90.20)	3.09 (1.10-8.71; 0.03)	2.14(0.63-7.22; 0.22)
	No	11 (3.40)	312 (96.60)	1:00	1:00
Sharing needles	Yes	1 (12.50)	7 (87.5%)	3.21 (0.37-27.71;0.29)	
	No	16 (4.30)	360 (95.70)	1:00	
CD_4 T cell count/µl	< 200	0 (0)	46 (100)		
	200-349	6 (9.84)	55 (90.16)	2.17 (0.72-653;0.16)	
	350-499	3 (2.72)	107 (97.28)	0.55 (0.14-2.15;0.39)	
	≥500	8 (4.79)	159 (95.21)	1:00	
ART status	ART naïve	3 (5.26)	54 (94.74)	1.24 (0.35-4.67;0.74)	
	On ART	14 (4.28)	313 (95.72)	1:00	
HIV/AIDS stage	I	11 (3.94)	268 (96.06)	1:00	
	II	4 (5.33)	71 (94.66)	1.37 (0.42-4.44;0.60)	
	III	1 (5.88)	16 (94.22)	1.52 (0.19-12.54;0.69)	
	IV	1 (7.69)	12 (92.31)	2.03 (0.24-1704;0.50)	
BMI (kg/m^2)	< 18.5	6 (4.55)	126 (95.44)	1.10 (0.36-2.85; 0.91)	
	18.5-24.9	10 (4.59)	208 (95.41)	1:00	
	25-29.9	1 (2.94)	33 (97.06)	1.59 (0.20-12.80; 0.66)	

DISCUSSION

Since the introduction of highly active antiretroviral therapy (HAART), AIDS-related mortality has declined significantly, improving the quality of life for HIV-positive individuals. However, end-stage liver disease due to co-infection with hepatotropic viruses has emerged as a major cause of morbidity and mortality in this population (12). Co-infection with viral hepatitis also increases the risk of ART-related hepatotoxicity (13, 14). Globally, HBV/HIV or HCV/HIV co-infections result in substantially higher liver-related morbidity and mortality compared to HIV mono-infection, with approximately one-third of HIV-related deaths attributed to liver disease in the context of HBV/HCV co-infection (15, 16).

In this study, the prevalence of HBV/HIV co-infection was 4.4% and no HCV and HIV or HBV, HCV and HIV co-infections were detected. This prevalence aligns with the World Health Organization's classification of intermediate HBV endemicity (2–7%) (2) and is comparable to reports from China (4.9%) (17), the USA (4.5%) (18), Nepal (4.4%) (19), Uganda (4.1%) (20), and Addis Ababa, Ethiopia (3.9%) (21). It is lower than rates reported in Gondar, Ethiopia (10.9%) (22) and other countries, including Nigeria (30.4%) (23), Italy (15.4%) (24), Iran (14.5%) (25), Gambia (12.2%) (26), and Japan (11.9%) (27), but higher than in Tanzania (1.2%) (28) and Mali (1.13%) (29). The observed variations in the prevalence of HBV infection across the different nations and study settings may reflect differences in socio-economic conditions, healthcare access, living standards, and vaccination coverage.

Although not statistically significant the HBV and HIV co-infection was more common among males (5.8%) than females (3.7%). This observation is consistent with findings from Kenya and Nigeria (30–32). The higher prevalence in male participants may be due to higher exposure to horizontal HBV transmission routes and culturally accepted multiple sexual partnerships in sub-Saharan Africa (33, 34).

Age was significantly associated with HBV co-infection, with the highest prevalence observed among participants over 60 years (AOR = 0.08; 95% CI: 0.01–0.67), possibly reflecting chronic infection acquired earlier in life. Similar age-related trends have been reported in Brazil and Taiwan (35, 36), though contrasting findings exist from Kenya and Nigeria, where younger adults exhibited higher prevalence (37, 38). Although marital status was not significantly associated with HBV infection, widowed, single, and divorced participants had higher co-infection rates than married individuals, possibly due to increased sexual risk behaviors, consistent with findings United Kingdom (39). Occupational status was also significantly associated with HBV infection ($p = 0.04$), suggesting that varying exposure risks may be influenced by socio-cultural and behavioral factors (40, 41).

No significant association was observed between HBV co-infection and CD4+ T-cell counts, aligning with studies from Europe and Nigeria (42, 43). No HBsAg positivity was detected among participants with severe immunosuppression (CD4 <200 cells/μL), potentially reflecting reduced exposure or increased caution in this group, although other studies report higher HBV prevalence at lower CD4 counts (44–48).

Unsafe injection practices remain an important global risk factor for HBV transmission, particularly in resource-limited settings where reusing injection equipment is common (49). In this study, approximately 10% of participants with a history of unsafe injections tested positive for HBsAg, consistent with reports from Ethiopia and WHO data (50, 51).

Multivariate logistic regression identified three independent predictors of HBV infection: history of tooth extraction, sexually transmitted diseases (STDs), and multiple sexual partners. Traditional tooth extraction using unsterilized instruments is common in sub-Saharan Africa and represents a recognized HBV transmission route (52, 53). Participants with a history of tooth extraction had over threefold higher odds of HBV infection (AOR = 3.17; 95% CI: 1.03–9.82), consistent with previous Ethiopian studies (54), though findings are mixed in other reports (55, 56).

A history of STDs was associated with a 3.5-fold increased risk of HBV infection (AOR = 3.53; 95% CI: 1.09–11.47), supporting evidence that ulcerative STDs and gonorrhea facilitate HBV transmission (57–59). Notably, participants reporting multiple sexual exposures had nearly tenfold higher odds of HBV infection (AOR = 9.68; 95% CI: 2.45–38.24), reinforcing the importance of sexual transmission as a key route in HIV-infected populations (60, 61).

CONCLUSIONS

This study demonstrated an intermediate prevalence of HBV and HIV co-infection. Tooth extraction, multiple sexual partners, and STDs were identified as independent predictors of HBV infection in HIV patients. These findings emphasize the continuing role of sexual transmission and unsafe traditional medical practices in HBV spread among HIV-positive populations. Given the increased risk of liver-related complications and ART-associated hepatotoxicity among co-infected individuals, routine HBV screening and timely linkage to care are essential. Public health interventions should include community awareness campaigns, promotion of safer sexual practices, discouragement of unsafe traditional medical procedures, and expansion of HBV vaccination among high-risk groups, particularly people living with HIV.

Data availability: All data supporting the findings of this study are included within the article.

Conflicts of interests

The authors declare no conflicts of interest.

Authors' contributions

AM, BD, AA, and DA contributed to study conception, experimental design, data interpretation, and critical manuscript review. BD and AA collected samples and relevant data, performed laboratory work, and drafted the manuscript. All authors read and approved the final manuscript.

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Oral Cancer Awareness among Patients Visiting Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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ABSTRACT

Background: Despite oral cancer being largely preventable and early detection significantly improving treatment outcomes, public awareness of its risk factors, early signs, and symptoms remains low, particularly in low-resource settings. In Ethiopia, late presentation is common, contributing to poor prognosis and increased morbidity. However, there is limited evidence regarding patients' knowledge and awareness of oral cancer in Addis Ababa. This gap in information hinders the development of targeted educational and preventive strategies.

Objectives: To assess the awareness and knowledge of oral cancer among patients visiting Tikur Anbessa Specialized Hospital (TASH) to inform community education programs and early detection initiatives.

Methods: An institution-based cross-sectional study was conducted among 221 adult patients attending the Department of Dentistry and Oral and Maxillofacial Surgery at TASH from May to June 2023. Data were collected using a structured interviewer-administered questionnaire assessing awareness and knowledge of oral cancer, including risk factors, signs, symptoms, and preventive measures. Knowledge was measured using a composite score, and participants scoring ≥ the mean (2.11) were classified as having good knowledge. Associations between socio-demographic factors and knowledge were first explored using Chi-square tests, followed by multivariable logistic regression to identify independent predictors of good knowledge, with $p < 0.05$ considered statistically significant.

Results: Among participants (mean age 37.1 ± 13.8 years; 60.2% female), 75.1% (n = 166) had never heard of oral cancer. Among those aware (24.9%, n = 55), the primary sources of information were media (58.2%, n = 32), while only 18.2% (n = 10) received information from health professionals. Cigarette smoking (69.2%) and alcohol consumption (55.2%) were the most recognized risk factors, whereas family history (38.4%), poor oral hygiene (46.6%), and advanced age (36.2%) were less frequently identified. Awareness of signs and symptoms was low, with less than 40% identifying non-healing ulcers (38.9%) or abnormal swelling (33.0%). Overall, knowledge was poor among most participants. Urban residence ($p = 0.0008$) and higher education ($p = 0.001$) were significantly associated with better knowledge; gender was not.

Conclusion: Awareness and knowledge of oral cancer were low, highlighting the need for targeted community education, patient counseling by health professionals, and integration of oral cancer information into routine dental care to improve early detection and reduce disease burden.

Keywords: Oral cancer, Awareness, Knowledge, Risk factors, Signs and symptoms, Ethiopia

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INTRODUCTION

Oral cancer (OC) is a malignant tumour affecting the anatomical structures of the oral cavity, including the lips, tongue, and other oral components (1). According to the American Joint Committee on Cancer (AJCC), the oral cavity is classified into seven sub-sites: lips, alveolar ridges, floor of the mouth, oral tongue (anterior two-thirds), retromolar trigone, and hard palate. Understanding these sub-sites is important for determining incidence patterns and lymphatic drainage. The prognosis of oral cancer varies depending on the anatomical site (2).

Globally, and particularly in developing countries, oral cancer contributes significantly to morbidity and mortality. Approximately 1.4 million new cases and 300,000 deaths (2.1% of all cancer-related deaths) are reported annually worldwide (3,4). Oral cancer is the eleventh most common malignancy globally (5).

Major risk factors for oral cancer include tobacco use, alcohol consumption, and human papillomavirus (HPV) infection. Other contributing factors are lifestyle behaviours, age, poor diet low in fruits and vegetables, and lack of physical activity (6-8). The signs and symptoms of oral cancer vary widely, ranging from early manifestations such as white and red patches on the oral mucosa to advanced symptoms, including persistent neck pain and voice changes. Intraoral findings may include non-healing ulcers, swellings, loosening of teeth, bleeding, and jaw pain. Functional impairments can include difficulty or pain during swallowing, speech difficulties, and reduced tongue mobility. Other manifestations include numbness of the tongue or lips, unexplained bleeding, neck swelling, fetor oris, altered dental occlusion, and sore throat (9).

The TNM staging system has historically provided clinicians with a reliable framework for prognostic assessment and management decisions in oral cancer (10). Additional pathological parameters, including histopathological grading according to WHO, presence of vascular and perineural invasion, extracapsular spread, and positive surgical margins, are also important prognostic indicators (11, 12). Surgery remains the mainstay of treatment for oral cancer, while primary and adjuvant radiotherapy and chemotherapy play significant roles in comprehensive management (13).

Although individuals can perform self-examinations of the oral cavity to detect lesions, and dentists are well-placed to identify early signs, evidence supporting the effectiveness of such assessments in reducing oral cancer mortality is limited. Nevertheless, it is recommended that dentists remain vigilant for potentially malignant disorders (PMDs) and oral cancer during routine clinical practice (14,15).

Enhancing public understanding of oral cancer risk factors, signs, and symptoms is critical for effective prevention. Increased awareness can lead to early detection and diagnosis, improving patient outcomes and prognosis. By reducing exposure to risk factors and recognizing early clinical signs, the prevalence and severity of oral cancer can be minimized (16,17).

Despite oral cancer being largely preventable and early detection playing a crucial role in improving prognosis and survival, public awareness of its risk factors, warning signs, and symptoms remains inadequate in many low- and middle-income countries. In Ethiopia, oral cancer is often diagnosed at advanced stages, contributing to poor treatment outcomes and increased morbidity and mortality. Limited public knowledge regarding major risk factors such as tobacco use, alcohol consumption, human papillomavirus infection, and delayed recognition of early lesions may hinder timely healthcare-seeking behavior. However, there is scarce evidence regarding patients' awareness and understanding of oral cancer in Addis Ababa. This knowledge gap makes it challenging to design targeted educational interventions and effective prevention strategies. Therefore, this study aims to assess the level of awareness and knowledge of oral cancer among patients visiting Tikur Anbessa Specialized Hospital, generating evidence to inform community education programs, early detection initiatives, and health policy measures to reduce the burden of oral cancer in Ethiopia.

MATERIALS AND METHODS

Study Design and Setting

An institution-based cross-sectional study was conducted at the Department of Dentistry and Oral and Maxillofacial Surgery (OMFS) Clinic, Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia, from May to June 2023. Tikur Anbessa is the largest referral hospital in Ethiopia, serving a diverse population from both urban and rural areas.

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This study aimed to assess patients' awareness and knowledge of oral cancer, including risk factors, signs, and symptoms, and to explore associations with socio-demographic characteristics.

Study Population

The study population included all adult patients (≥18 years) attending the Dentistry and OMFS Clinic during the study period. Patients who were critically ill, unable to communicate, or unwilling to participate were excluded.

Sample Size Determination

The sample size was calculated using the single population proportion formula:

$$n = \frac{Z^2 \cdot p \cdot (1 - p)}{d^2}$$

Where:

□ = required sample size

□ = standard normal value at 95% confidence interval (1.96)

□ = estimated proportion of patients with adequate knowledge of oral cancer. Since no prior data were available in Addis Ababa, we assumed p = 50% to maximize sample size.

□ = margin of error (5%)

Considering the finite population of patients attending the clinic during the study period (approximately 400), the sample size was adjusted using the finite population correction formula:

$$n_{adj} = \frac{n}{1 + (n/N)} = \frac{384}{1 + (384/400)} \approx 196$$

Adding 10% for non-response, the final sample size was approximately 216 participants. A total of 221 patients were ultimately recruited, meeting the calculated sample size.

Sampling Technique

A systematic random sampling method was employed. The expected number of adult patients visiting the clinic during the study period was estimated from the hospital registry. The sampling interval □ was calculated as:

$$k = \frac{N}{n} = \frac{400}{221} \approx 2$$

The first participant was selected randomly from the first two patients, and every second patient thereafter was included until the required sample size was achieved. Patients meeting the inclusion criteria but unwilling to participate were skipped, and the next eligible patient was selected.

Data Collection Instrument and Procedure

Data were collected using a structured interviewer-administered questionnaire developed from previous studies and international guidelines on oral cancer awareness (18–20). The questionnaire had four main sections: socio-demographic characteristics, substance use habits, awareness and sources of information, and knowledge of oral cancer risk factors and signs/symptoms. The instrument was pre-tested on 10% of the sample at a different clinic, and minor modifications were made to improve clarity.

Measurement of Knowledge

Knowledge of oral cancer was assessed using a composite scoring system. Correct responses for risk factors and signs/symptoms were summed. Participants scoring greater than or equal to the mean (2.11) were classified as having good knowledge, while those scoring below the mean were classified as having poor knowledge.

Data Management and Analysis

Data were coded, entered, and analyzed using SPSS version 26.0. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were used to summarize socio-demographic characteristics, substance use habits, and awareness and knowledge of oral cancer.

Associations between socio-demographic factors and knowledge of oral cancer were first assessed using Chi-square tests. Factors with $p < 0.2$ in bivariate analysis were further included in a multivariable logistic regression model to identify independent predictors of good knowledge. Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were reported. Statistical significance was set at $p < 0.05$.

Ethical Considerations

Ethical approval was obtained from the Addis Ababa University, College of Health Sciences Institutional Review Board. Written informed consent was obtained from all participants. Confidentiality and privacy were maintained throughout the study, and participation was voluntary.

RESULTS

Socio-demographic Characteristics

A total of 221 patients participated in the study, with a mean age of 37.1 ± 13.8 years. The majority were female (60.2%,

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n = 133), resulting in a female-to-male ratio of 1.51:1. The largest age group was 18–30 years (38.9%, n = 86), followed by 31–40 years (29.0%, n = 64), 41–50 years (17.6%, n = 39), 51–60 years (9.5%, n = 21), and >60 years (5.0%, n = 11).

Most participants resided in urban areas (86.4%, n = 191). Regarding occupation, 57.9% (n = 128) were employed, 25.8% (n = 57) were unemployed, and 16.3% (n = 36) were students. In terms of education, 52.0% (n = 115) had primary or secondary education, 40.7% (n = 90) had higher education, and 7.2% (n = 16) were unable to read or write (Table 1).

Table 1: Socio-demographic characteristics of patients who visited the Dentistry and OMFS Clinic at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, May–June 2023 (n = 221)

Characteristics	Frequency (n)	Percentage (%)
Age groups (years)*		
18-30	86	38.9
31-40	64	29.0
41-50	39	17.6
51-60	21	9.5
> 60	11	5.0
Gender		
Male	88	39.8
Female	133	60.2
Residence		
Urban	191	86.4
Rural	30	13.6
Education		
Unschooled	16	7.2
Primary & secondary	115	52.0
University	90	40.7
Occupation		
Unemployed	57	25.8
Employed	128	57.9
Student	36	16.3

**Mean age = 37.1 ± 13.8 years.*

Substance Use and Sources of Information

Regarding substance use habits, the majority of participants, 65.2% (n = 144), reported no history of substance use. Among users, 10.0% (n = 22) were khat chewers, 6.3% (n = 14) were cigarette smokers, 14.0% (n = 31) were alcohol consumers, 0.9% (n = 2) were shisha smokers, and 3.6% (n = 8) reported using multiple substances.

Regarding awareness of oral cancer, 75.1% (n = 166) had never heard of it. Among those aware (24.9%, n = 55), the primary sources of information were media (58.2%, n = 32), including television, radio, the internet, social media, newspapers, and magazines. Only 18.2% (n = 10) obtained information through direct contact with health professionals, whereas the remaining 23.6% (n = 13) learned about oral cancer from other individuals including friends, family, and community.

Knowledge of Oral Cancer Risk Factors and Signs and Symptoms

Table 2 presents participants' awareness of oral cancer risk factors and signs and symptoms. Recognition of risk factors was variable. Cigarette smoking (69.2%) and alcohol consumption (55.2%) were most frequently correctly identified. Only 38.4% of respondents considered a family history of oral cancer to be a risk factor, while a comparable proportion (35.8%) did not believe it was associated. Excessive exposure to sunlight was identified as a risk factor by only 22.1% of participants. Similarly, 36.2% recognized advanced age as a risk factor, while 33.5% disagreed and the remainder were unsure. Poor oral hygiene was identified as a risk factor by 46.6% of respondents, and 38.4% considered chronic trauma a risk factor. Fewer participants recognized sedentary lifestyle (22.2%) and consumption of hot and spicy foods (17.6%) as potential risk factors. Notably, 13.1% of respondents believed that demonic attack could be a risk factor for oral cancer, although the majority (71.5%) correctly did not consider it a risk factor (**Table 2).**

Awareness of oral cancer signs and symptoms was generally low. Non-healing sores or ulcers that bleed were identified by 38.9%, abnormal swelling by 33.0%, neck lumps by 26.7%, continuous pain by 23.5%, white or red plaques by 24.9%, unexplained tooth loss by 19.9%, bleeding by 20.8%, loss of taste by 20.4%, burning sensation by 19.9%, numbness by 17.2%, and difficulty chewing or swallowing by 17.2% (**Table 2**).

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Table 2: Awareness of oral cancer risk factors and signs and symptoms among patients who visited the Dentistry and OMFS Clinic at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, May–June 2023 (n = 221)

Risk factors	Correctly identified (n)	% Correct
Cigarette smoking	153	69.2%
Alcohol drinking	122	55.2%
Family history of oral cancer	85	38.4%
Excessive sunlight exposure	49	22.1%
Advanced age	80	36.2%
Poor oral hygiene	103	46.6%
Chronic trauma	85	38.4%
Sedentary lifestyle	49	22.2%
Hot and Spicy foods	39	17.6%
Demonic attack*	29	13.1%
Signs and symptoms		
Loss of taste	45	20.4%
Bleeding	46	20.8%
Burning Sensation	44	19.9%
Numbness	38	17.2%
Difficulty in chewing or swallowing	55	24.9%
Abnormal swelling	73	33.0%
Non-healing sore or ulcer that bleeds	86	38.9%
Loosening or unexplained loss of teeth	44	19.9%
Continuous pain	52	23.5%
White or red patch	55	24.9%
Thickening/ lump in the neck	59	26.7%

*Incorrect responses, such as demonic attack, are included to show misconceptions.

Factors associated with Knowledge

Overall, the majority of participants had poor knowledge of oral cancer. In bivariate and multivariable analysis, urban residence and higher education were significant independent predictors of good knowledge (p = 0.0008 and p = 0.001, respectively), while gender was not significantly associated. The highest proportion of participants with good knowledge was observed in the 18–30 years age group.

Among male participants, 18.1% (n = 40) demonstrated good knowledge, while 21.7% (n = 48) had poor knowledge. Among females, 27.1% (n = 60) had good knowledge and 33.0% (n = 73) had poor knowledge. The difference in knowledge by gender was not statistically significant (P = 0.96). Of participants residing in urban areas, 35.3% (n = 78) had good knowledge, whereas 51.1% (n = 113) had poor knowledge. Among rural residents, 9.9% (n = 22) showed good knowledge, and 3.6% (n = 8) had poor knowledge. This association was statistically significant (P = 0.0008). The highest proportion of participants with good knowledge was in the 18–30 years age group (19%), followed by 31–40 years (13.1%), 41–50 years (6.3%), 51–60 years (4.1%), and >60 years (1.8%). Among those with poor knowledge, 19.9% were aged 18–30 years, 15.8% were 31–40 years, 11.3% were 41–50 years, 5.4% were 51–60 years, and 3.2% were >60 years. Among participants with good knowledge, 5.9% were unschooled, 26.7% had primary or secondary education, and 12.7% had higher education. In contrast, among those with poor knowledge, 1.4% were unschooled, 25.3% had primary or secondary education, and 28.1% had higher education. The association between education level and knowledge was statistically significant (P = 0.001).

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DISCUSSION

This study was conducted at Addis Ababa University, College of Health Sciences, Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia. To the best of our knowledge, this is the first institution-based study in Ethiopia assessing awareness of oral cancer risk factors, signs, and symptoms among dental patients. Oral cancer remains a significant global public health problem, with increasing incidence in many regions worldwide (4,8). Despite advances in diagnosis and treatment, the five-year survival rate remains approximately 50%, largely due to late-stage presentation and delayed diagnosis (2,18). Early detection and preventive strategies in dental practice are therefore essential for improving outcomes (1,14).

In the present study, females constituted 60.2% of participants, and the largest age group was 18–30 years (38.9%). A similar female predominance has been reported in studies conducted in India and other regions (19, 20), although age distribution varies across populations. Differences in demographic structure may be attributed to variations in healthcare -seeking behavior, study design, and sampling methods.

Most participants (86.4%) were urban residents, which reflects the hospital's location in the capital city. Educational attainment was relatively high, yet awareness of oral cancer was low. Notably, 75.1% of respondents had never heard of oral cancer. Comparable low awareness levels have been reported in Yemen, Portugal, and Tehran, Iran (21-23). These findings suggest that formal education alone does not necessarily translate into disease-specific awareness, highlighting gaps in public health communication.

Substance use is a major modifiable risk factor for oral cancer. Cigarette smoking was identified as a risk factor by 69.2% of participants. Although lower than the awareness reported in Sudan (24), this finding is higher than reports from Beijing, China (25). Tobacco use is a well-established etiological factor for oral and oropharyngeal cancers (6,26). However, public perception often associates smoking primarily with lung cancer rather than oral malignancies. Public health strategies such as taxation, advertising restrictions, and anti-smoking campaigns remain crucial in reducing tobacco-related cancers (27).

Alcohol consumption was identified as a risk factor by 55.2% of participants, consistent with findings from Australian dental patients (28). Alcohol acts synergistically with tobacco to increase oral cancer risk (5,29). However, awareness of other risk factors such as chronic trauma, advanced age, and sun exposure was relatively low, indicating incomplete understanding of multifactorial etiology.

Awareness of early signs and symptoms was also limited. Only 38.9% recognized a non-healing ulcer as a warning sign, and fewer than one-quarter identified red or white patches as potential indicators of malignancy. Similar findings have been reported in Brazil and Malaysia (30,31). Given that many oral cancers arise from potentially malignant disorders such as leukoplakia and erythroplakia (9,12), limited recognition of early symptoms may contribute to delayed healthcare seeking and poor prognosis.

Mass media was the primary source of information (66.7%), consistent with evidence that media-based interventions can improve cancer awareness (24,25). However, only 6.2% reported receiving information from healthcare professionals. Considering the critical role of dentists in early detection and opportunistic screening (14,15), these finding highlights missed opportunities for patient education within clinical settings.

Socio-demographic factors influenced knowledge levels. Education and residence were significantly associated with good knowledge ($P = 0.001$ and $P = 0.0008$, respectively). Similar associations have been observed in Malaysia and Iran (32,33). Younger participants demonstrated relatively better awareness, which may reflect greater exposure to digital and social media platforms. Gender differences were not statistically significant in this study, although other studies have reported higher awareness among females (34-38).

Overall, the findings demonstrate persistently low awareness of oral cancer despite its preventable nature. Since early detection significantly improves survival outcomes (7,18), strengthening public education and integrating routine oral cancer screening into dental practice are imperative.

Strength and Limitations

This study has several limitations. First, it was conducted in a single tertiary referral hospital in an urban setting, which may limit generalizability to rural populations and the broader Ethiopian community. Second, the cross-sectional design

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precludes establishing causal relationships between socio-demographic factors and knowledge levels. Third, self-reported responses may be subject to recall bias or social desirability bias. Finally, the use of a mean score to dichotomize knowledge may not fully capture the complexity of awareness levels.

Despite these limitations, the study provides important baseline data for Ethiopia and highlights critical gaps in oral cancer awareness.

CONCLUSION AND RECOMMENDATIONS

This study revealed a generally low level of awareness regarding oral cancer among patients attending the Dentistry and Oral and Maxillofacial Surgery Clinic at Tikur Anbessa Specialized Hospital. Knowledge of major risk factors, such as tobacco and alcohol use, was moderate; however, awareness of other important risk factors and early warning signs was limited. A substantial proportion of participants had never heard of oral cancer, which may contribute to delayed health-seeking behavior and late-stage diagnosis. Educational status and place of residence were significantly associated with knowledge levels, highlighting disparities in access to health information. These findings indicate an urgent need for strengthened public health education and preventive strategies to improve early detection and reduce morbidity and mortality associated with oral cancer.

Based on the findings of this study, comprehensive public awareness initiatives should be implemented to improve knowledge of oral cancer risk factors and early signs. Mass media platforms, including television, radio, and social media, should be strategically utilized, as they were identified as the primary source of information among participants. In addition, healthcare professionals, particularly dentists and physicians, should actively incorporate oral cancer education and routine opportunistic screening into daily clinical practice. Community-based health education programs should be expanded, particularly targeting rural populations and individuals with lower educational attainment. Integrating oral cancer awareness into school health programs and university curricula may further promote early knowledge and preventive behaviors. Finally, further multicenter and community-based research is recommended to assess awareness levels across different regions of Ethiopia and to evaluate the effectiveness of educational interventions.

Author Contribution

All authors contributed equally to the conception, design, data collection, analysis, interpretation of results, and drafting of the manuscript. All authors have read and approved the final version for submission and agree to be accountable for all aspects of the work.

Conflict of Interest

The authors declare no potential conflicts of interest related to this research, authorship, or publication.

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Data Availability

The data supporting the findings of this study are available upon request.

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Prevalence and Antimicrobial Resistance Patterns of Gram-Negative Bacteria at Amhara Public Health Institute, Northwest Ethiopia: A Six-Year Retrospective Study

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ABSTRACT

Background: The global rise of multidrug-resistant Gram-negative bacteria presents a major public health challenge, particularly in low- and middle-income countries. Limited antimicrobial resistance surveillance data in Ethiopia hampers evidence-based treatment and policy decisions.

Objective: This study assessed the prevalence and antimicrobial resistance patterns of Gram-negative bacteria isolated over six years (2018–2024) at the Amhara Public Health Institute (APHI), Northwest Ethiopia.

Methods: An institution-based retrospective study was conducted using archived microbiology laboratory data from January 2018 to December 2024. All culture-confirmed Gram-negative bacterial isolates with complete antimicrobial susceptibility test results were included. Antimicrobial susceptibility test was performed using the Kirby–Bauer disk diffusion method according to the CLSI guidelines. Data were analyzed SPSS version 26 software. Chi-square tests were used to assess the associations between outcome and demographic variables. Statistical significance was declared at $p < 0.05$.

Results: A total of 350 Gram-negative isolates were analyzed. Escherichia coli (29.4%, 95% CI: 24.6–34.2) and Klebsiella spp. (27.7%, 95% CI: 23.0–32.4) were the predominant isolates. Overall, 53.2% of isolates were classified as MDR. Highest resistance rates were observed against chloramphenicol (97.3%), sulfonamides (80.3%), and ampicillin (82.4%). The isolates showed the lowest resistance against Carbapenems (24.9%). Age ($\chi^2 = 106.69$, $p < 0.001$) and sex ($\chi^2 = 12.86$, $p = 0.045$) were significantly associated with multidrug resistance bacterial infection.

Conclusion: More than half of Gram-negative isolates were multidrug resistant in the study area. Strengthening AMR surveillance, antimicrobial stewardship programs, and routine culture-based diagnosis is urgently required.

Keywords: Multidrug resistance; Gram-negative bacteria; Antimicrobial resistance; Ethiopia; Bahir Dar.

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INTRODUCTION

Antimicrobial resistance (AMR) among Gram-negative bacteria has increased substantially over the past decade and is now recognized as a major global public health threat (1). These organisms have developed resistance to multiple commonly used antibiotics, resulting in the emergence of so-called "superbugs," which significantly complicate the treatment of bacterial infections (2). Multidrug resistance (MDR) is defined as resistance to at least one antimicrobial agent in three or more antibiotic classes, often mediated by the acquisition of multiple resistance genes and mechanisms (3).

Gram-negative bacteria, particularly members of the *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species, as well as non-fermenting organisms including *Pseudomonas aeruginosa* and *Acinetobacter* species, are among the leading causes of MDR infections worldwide (4). According to the first comprehensive global assessment, bacterial AMR was associated with approximately 4.95 million deaths in 2019, including 1.27 million deaths directly attributable to resistant infections. The burden of AMR is disproportionately higher in low- and middle-income countries due to limited diagnostic capacity, weak surveillance systems, and widespread inappropriate antibiotic use (5).**Top of Form**

Bottom of Form

Evidence from developing countries indicates that Gram-negative bacteria exhibit high resistance rates to commonly prescribed antibiotics, thereby reducing treatment effectiveness and increasing morbidity, mortality, and healthcare costs (6). In Ethiopia, a systematic review and meta-analysis reported a pooled MDR prevalence of 70.5%, highlighting the magnitude of the problem and the urgent need for effective interventions (7). To address this global health threat, the World Health Organization (WHO) recommends strengthening antimicrobial stewardship programs, enhancing surveillance systems, and promoting rational antibiotic use (8).

Routine epidemiological surveillance of antimicrobial resistance is essential to monitor trends, guide empirical treatment, and inform infection prevention and control strategies. Establishing robust surveillance systems and generating reliable local data are critical components of national and global AMR control efforts (9). However, in many healthcare settings in Ethiopia, comprehensive data on the prevalence and antimicrobial susceptibility patterns of Gram-negative bacterial isolates remain limited.

The lack of local epidemiological evidence hinders evidence-based clinical decision-making, contributes to inappropriate empirical antibiotic use, and accelerates the development and spread of resistance. Furthermore, antimicrobial resistance surveillance systems remain insufficient in many parts of the country. Generating institution-specific and region-specific AMR data is therefore essential to support clinical management, guide antimicrobial stewardship programs, and inform national AMR containment strategies. Therefore, this study aimed to determine the prevalence and antimicrobial resistance patterns of Gram-negative bacterial isolates tested from 2018 to 2024 at the Amhara Public Health Institute, Bahir Dar, Northwest Ethiopia.

MATERIALS AND METHODS

Study setting, design and period

An institution-based retrospective cross-sectional study was conducted at the Amhara Public Health Institute (APHI), located in Bahir Dar, Amhara National Regional State, Northwest Ethiopia. The Amhara Public Health Institute serves as a regional reference laboratory, receiving clinical specimens from hospitals and health facilities across Bahir Dar and surrounding areas. The bacteriology reference laboratory performs bacterial identification and antimicrobial susceptibility testing following standard microbiological procedures. The study analyzed archived microbiology laboratory data of Gram-negative bacterial isolates obtained from clinical specimens collected between January 2018 and December 2024.

Study population and sample size

The study included all clinical specimens that yielded Gram-negative bacterial isolates and had complete antimicrobial susceptibility testing (AST) results during the study period. A census sampling method was used, and all eligible laboratory records meeting the inclusion criteria were included in the analysis.

Inclusion and Exclusion Criteria

Laboratory-confirmed Gram-negative bacterial isolates with

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complete antimicrobial susceptibility testing results, and records within the study period (2018–2024) were included in this study. While those incomplete laboratory records and missing antimicrobial susceptibility results were excluded.

Data collection procedures

Socio-demographic and laboratory data were retrieved from archived laboratory records at the Microbiology Reference Laboratory of APHI using a structured data extraction format developed in Microsoft Excel. The extracted variables included age, sex, year of testing, type of clinical specimen, bacterial isolates, and antimicrobial susceptibility results.

Clinical specimens used for isolation of the Gram-negative bacteria were urine, blood, sputum, wound/pus, cerebrospinal fluid, body fluids, ear discharge, eye discharge, throat swabs, and other relevant specimen types. To ensure confidentiality, a unique identification code was assigned to each patient record, and no personal identifiers were used.

Bacterial identification and antimicrobial susceptibility test

Clinical specimens were processed using the routine standard microbiological techniques at the Amhara Public Health Institute. Specimens were inoculated onto appropriate culture media and incubated at 37 °C. Bacterial isolates were identified based on colony morphology, Gram reaction, and biochemical tests.

Antimicrobial susceptibility test was performed using the Kirby–Bauer disk diffusion method on Mueller-Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI M100) guidelines. Bacterial suspensions were prepared and adjusted to 0.5 McFarland turbidity standard and inoculated onto Mueller–Hinton agar plates. Antibiotic disks were applied, and plates were incubated at 37 °C for 16–18 hours. The diameter of inhibition zones was measured and interpreted as susceptible, intermediate, or resistant according to the CLSI criteria.

The following antibiotics were tested: Amoxicillin/clavulanic acid (20/10 µg), Ampicillin (10 µg), Cefepime (30 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Tobramycin (10 µg), Trimethoprim–sulfamethoxazole (1.25/23.75 µg), Nitrofurantoin (300 µg), Imipenem (10 µg), Meropenem (10 µg), and Tetracycline (15 µg).

Multidrug resistance (MDR) was defined as resistance to at least one antibiotic agent in three or more antimicrobial classes.

Data management and Analysis

Data were checked for completeness, coded, and entered into Epi Info version 7.2.5, and then exported to SPSS version 26.0 for analysis. Descriptive statistics, including frequencies, percentages, and proportions, were used to summarize bacterial prevalence and antimicrobial resistance patterns. The prevalence of multidrug resistance was calculated as the proportion of isolates resistant to at least one antibiotic in three or more antimicrobial classes. The Chi-square test was used to assess associations between bacterial isolates and demographic variables such as age and sex of patients. A p-value of less than 0.05 was considered statistically significant.

Ethical considerations

Ethical approval was obtained from the Institutional Review Board (IRB) of the Amhara Public Health Institute (Protocol No: IRB NoH/R/T/T/D/07/74). Permission was also obtained from the APHI laboratory diagnostic directorate.

The requirement for informed consent was waived due to the retrospective nature of the study. All data were anonymized, and confidentiality was maintained throughout the study. The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Prevalence of Gram-negative bacterial isolates

A total of 350 Gram-negative bacterial isolates were identified from the different clinical specimens processed. Overall, *Escherichia coli* was the most frequently isolated organism, accounting for 29.4% (103/350; 95% CI: 24.6–34.2) of all isolates. This was followed by *Klebsiella* spp., which comprised 27.7% (97/350; 95% CI: 23.0–32.4). *Acinetobacter baumannii* and *Enterobacter* spp. accounted for 12.6% (44/350; 95% CI: 9.1–16.1) and 12.0% (42/350; 95% CI: 8.6 –15.4) of isolates, respectively.

Pseudomonas aeruginosa represented 10.0% (35/350; 95% CI: 6.9–13.1) of the total isolates. Lower prevalence was observed for *Proteus* spp., accounting for 5.1% (18/350; 95% CI: 2.8–7.4), and *Citrobacter* spp., which comprised 3.1% (11/350; 95% CI: 1.3–5.0) of isolates (**Table 1**).

Table 1: Proportion of Gram-negative bacteria isolated at Amhara Public Health Institute, 2018 -2024

Bacterial isolates	Frequency	Percentage	95% CI
A. baumannii	44	12.6	9.1 – 16.1
Citrobacter spp.	11	3.1	1.3 – 5.0
E. coli	103	29.4	24.6 – 34.2
Enterobacter spp.	42	12.0	8.6 – 15.4
Klebsiella spp.	97	27.7	23.0 – 32.4
Proteus spp.	18	5.1	2.8 – 7.4
P. aeruginosa	35	10.0	6.9 – 13.1

Distribution of the Gram-negative bacterial isolates by age group and sex

The distribution of Gram-negative bacterial isolates varied significantly across different age groups (χ^2 = 106.69, p < 0.001). *Klebsiella* spp. and *Enterobacter* spp. were more frequently isolated among children under five years of age, accounting for 52.6% and 35.7% of isolates in this age group, respectively. *Acinetobacter baumannii* was most common among individuals aged 15–20 years (27.3%). *Proteus* spp. and *Pseudomonas aeruginosa* were predominantly isolated from patients aged 20–50 years, representing 72.2% and 65.7% of isolates in this age group, respectively. In contrast, *Escherichia coli* was more frequently isolated among patients aged above 50 years, accounting for 32.0% of isolates in this age category.

The distribution of Gram-negative bacterial isolates also showed a statistically significant association with sex (χ^2 = 12.86, p = 0.045). *Escherichia coli* was more commonly isolated among female patients, accounting for 56.3% of isolates. In contrast, *Proteus* spp. (77.8%), *Pseudomonas aeruginosa* (60.0%), and *Acinetobacter baumannii* (54.5%) were more frequently isolated from male patients (**Table 2**).

Table 2: Distribution of Gram-Negative bacterial isolates across age and sex of study participants at Amhara Public Health Institute, 2018 -2024

Variables	Isolated organisms							
	***A. baumannii* (n=44)**	***Citrobacter* spp. (n=11)**	***E. coli* (n=103)**	***Enterobacter* spp. (n=42)**	***Klebsiella* spp. (n=97)**	***Proteus* spp. (n=18)**	***P. aeruginosa* (n=35)**	**χ^2 (p-value)**
Age in years								89.6 (< 0.001)
< 5	18 (40.9%)	1 (9.1%)	15(14.6%)	23 (54.8%)	68 (70.1%)	1 (5.6%)	7 (20.0%)	
> 50	9 (20.5%)	2 (18.2%)	26(25.2%)	2 (4.8%)	2 (2.1%)	2 (11.1%)	3 (8.6%)	
20 – 50	12 (27.3%)	6 (54.5%)	51(49.5%)	12(28.6%)	17(17.5%)	12(66.7%)	18(51.4%)	
5 to 20	5(11.4%)	2(18.2%)	11(10.7%)	5(11.9%)	10(10.3%)	3(16.7%)	7(20.0%)	
Sex								14.8 (0.022)
Female	17(38.6%)	6(54.5%)	53(51.5%)	17(40.5%0	36(37.1%)	3(16.7%)	10(28.6%)	
Male	27(61.4%)	5(45.5%)	50(48.5%)	25(59.5%)	61(62.9%)	15(83.3%)	25(71.4%)	

Antimicrobial resistance patterns of Gram-negative bacteria

The antimicrobial resistance patterns varied across antibiotic classes. Overall, high resistance rates were observed against commonly used antibiotics. The highest resistance rates were observed against Chloramphenicol (97.3%), Ampicillin (82.4%), and Sulfonamides (80.3%), Cephalosporins (48.6–67.9%), Fluoroquinolones (42.5%), and Tetracycline (36.2%). Relatively, lower resistance was observed for Aminoglycosides (30.0%) and Carbapenems (24.9%) (**Table 3**).

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Table 3: Multi drug resistance of Gram-negative bacteria isolated at Amhara Public Health Institute, 2018 -2024

Antibiotic Class	Antibiotics	*A. baumannii*	*Citrobacter Spp.*	*E. aerogenes*	*E. Coli*	*Proteus spp.*	*Pseudomonas Spp.*	*Klebsiella Spp.*	Total
Penicillin	Amp	66.7%	81. %8	80%	70.8%	100%	100%	76.9%	82.4%
	AMC	83.3%	54. %5	100%	66.7%	31.5%	75%	84.6%	79.4%
Phenicol	CH	100 %	70%	100%	91.7%	100%	100%	92.3%	97.3%
Aminoglycosides	TOB	50 %	54.5%	20%	33.3%	54.6%	25%	69.2%	32.9%
	GN	50 %	54.4%	20%	20.8%	35.4%	25%	69.2%	26.7%
	CTR	50 %	63.6%	40%	41.7%	53.1%	50%	69.2%	47.4%
Cephalosporin	Cefta	50 %	72.7%	20%	45.8%	92.3%	50%	61.5%	37.9%
	Cefox	33.3%	68%	20%	29.2%	72%	50%	30.8%	32.8%
Fluoroquinolones	CIP	33.3 %	54.5%	100%	62.5%	40.7%	100%	76.9%	73.2%
Sulfonamides	CoT	100 %	72.7%	100%	70.8%	78.5%	75%	69.2%	80.3%
Nitrofurans	F	100 %	76%	20%	33.3%	68%	86%	84.6%	39.7%
Tetracycline	TE	66.7%	72.7%	60%	54.2%	76.9%	70%	56%	36.2%
Carbapenem	IMP	83.3%	63.6%	20%	45.8%	21.6%	20%	21%	24.9%

Amp: Ampicillin, AMC: Amoxicillin-clavulanic acid, CH: Chloramphenicol, TOB: Tobramycin, GN: Gentamicin. CTR: Ceftriaxone, Cefta: Ceftazidime, Cefox: Cefotaxime, CIP: Ciprofloxacin, CoT: Cotrimoxazole, F: *Nitrofurantoin*, TE: tetracycline, IMP: Imipenem.

DISCUSSION

The present study assessed the prevalence and antimicrobial resistance patterns of Gram-negative bacteria isolated from clinical specimens collected over a six-year period (2018–2024) at the Amhara Public Health Institute, Northwest Ethiopia. In this study, *Escherichia coli* was the most frequently isolated organism, accounting for 29.4% (95% CI: 24.6–34.2) of all isolates, followed by *Klebsiella* spp. (27.7%, 95% CI: 23.0–32.4). This finding is consistent with previous studies conducted in Ethiopia, which reported *E. coli* and *Klebsiella* spp. as the most predominant Gram-negative pathogens (10–13). The slight variation in proportions across studies may be attributed to differences in study population, specimen types, healthcare settings, and sample size.

Age-specific distribution showed that *Klebsiella* spp. and *Enterobacter* spp., followed by *Acinetobacter baumannii*, were more prevalent among children under five years of age. This finding aligns with previous reports indicating *Klebsiella pneumoniae* as one of the leading multidrug-resistant pathogens among pediatric populations (14). Among adults, *Proteus* spp. were the most frequently isolated organisms, which is comparable to findings from India reporting higher prevalence of *Proteus* species among adult populations, particularly in middle-aged and older individuals (15).

Sex-based analysis revealed that *E. coli* was the most prevalent organism among females, followed by *Klebsiella* spp., whereas *Proteus* spp. were the predominant isolates among males. This finding is consistent with a study conducted in Brazil that reported higher prevalence of *E. coli* among females and *Proteus mirabilis* among males (16). These differences may reflect variations in anatomical, physiological, and behavioral risk factors influencing infection susceptibility.

The present study also revealed a substantial burden of multidrug resistance among Gram-negative bacteria, with an overall MDR prevalence of 53.2%. This indicates that more than half of the isolates were resistant to at least three classes of

antibiotics, highlighting the growing challenge of antimicrobial resistance in the study setting. This finding is consistent with a systematic review conducted in Ethiopia, which reported MDR prevalence ranging from 30% to 85% among bacterial pathogens (17). These findings emphasize the increasing limitations of empirical antibiotic therapy and underscore the importance of routine antimicrobial susceptibility testing. Furthermore, these results align with global evidence indicating that Africa bears a disproportionate burden of antimicrobial resistance due to limited diagnostic capacity, unregulated antibiotic use, and resource constraints (5).

Analysis of antibiotic resistance patterns showed that Gram-negative bacteria exhibited high resistance to commonly used antibiotics. The highest resistance rates were observed for phenicol (97.3%), sulfonamides (80.3%), and penicillin (81%). Resistance to specific antibiotics was particularly high for chloramphenicol (97.3%), ampicillin (82.4%), amoxicillin-clavulanate (79.4%), ciprofloxacin (73.2%), and ceftriaxone (47.4%). In contrast, relatively lower resistance rates were observed for carbapenems (24.9%), aminoglycosides (30%), tetracyclines (36.2%), and nitrofurantoin (39.7%). These findings are consistent with studies conducted in Ethiopia, Tanzania, and Rwanda, which reported high resistance to commonly used antibiotics and relatively preserved effectiveness of carbapenems and aminoglycosides (18–20).

Organism-specific analysis showed that *E. coli* exhibited high resistance to phenicol and sulfonamides, moderate resistance to penicillin, fluoroquinolones, and tetracyclines, and lower resistance to carbapenems and aminoglycosides. Similar resistance patterns have been reported in studies conducted in China and other regions (21). *Klebsiella* spp. demonstrated high resistance to phenicol, fluoroquinolones, and penicillin, moderate resistance to cephalosporins and sulfonamides, and relatively low resistance to carbapenems. These findings are consistent with previous reports from Ethiopia (22).

A. baumannii exhibited extremely high resistance to multiple antibiotic classes, including complete resistance to phenicol, sulfonamides, and nitrofurantoin, and high resistance to carbapenems. These findings are consistent with studies conducted in Ethiopia, Nigeria, and Saudi Arabia, which reported widespread multidrug resistance among *Acinetobacter* species (23–26).

Similarly, *Citrobacter* spp. showed high resistance to sulfonamides, nitrofurans, and tetracyclines, with moderate resistance to penicillin, cephalosporins, and fluoroquinolones. These findings are supported by studies conducted in Ethiopia, Bangladesh, and systematic reviews reporting high resistance among *Citrobacter* isolates (27–29). *Enterobacter aerogenes* demonstrated extremely high resistance to phenicol, fluoroquinolones, and sulfonamides, but relatively low resistance to carbapenems and aminoglycosides, consistent with previous findings (30).

Proteus spp. exhibited high resistance to phenicol, cephalosporins, and sulfonamides, while maintaining relatively lower resistance to carbapenems and aminoglycosides. These findings align with studies conducted in Egypt and other regions (31). Likewise, *Pseudomonas* spp. showed high resistance to phenicol, fluoroquinolones, and penicillin, with lower resistance to carbapenems and aminoglycosides, consistent with reports from Ethiopia and Iraq (32). Overall, the high prevalence of multidrug resistance and widespread resistance to commonly used antibiotics observed in this study highlight the growing challenge of antimicrobial resistance in the study setting. The relatively lower resistance observed for carbapenems and aminoglycosides suggests that these antibiotics remain important treatment options; however, their use should be carefully monitored to prevent further resistance development.

Limitations of the study

This study has several limitations. First, due to its retrospective design, important clinical variables such as prior antibiotic use, hospitalization history, comorbidities, clinical outcomes, and infection source were not available. Second, the study was conducted at a single public health institute, which may limit the generalizability of the findings to other regions of Ethiopia.

CONCLUSION AND RECOMMENDATION

This study demonstrated a high prevalence of multidrug-resistant Gram-negative bacteria among clinical isolates. The most frequently isolated organisms were *Escherichia coli*, *Klebsiella* spp., and *Acinetobacter baumannii*. More than half of the isolates were multidrug resistant, with high resistance observed against commonly used antibiotics such as chloramphenicol, sulfonamides, and penicillin. In contrast,

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the isolates showed relatively lower resistance to carbapenems and aminoglycosides.

These findings highlight the urgent need to strengthen antimicrobial resistance surveillance, promote routine culture and susceptibility testing, and implement antimicrobial stewardship programs.

Authors' contributions

AA conceived and designed the study, performed the data analysis, and drafted the manuscript. MG, GB, and DS contributed to data analysis, interpretation of the findings, and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Declarations

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Availability of Data and Materials

All relevant data generated or analyzed during this study are included in this published article. The original dataset is available from the corresponding author upon reasonable request.

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