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Astrovirus infections in East Africa: Burden, diversity, and public health implications

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Editorial

Gastroenteritis remains a major global health concern, causing an estimated 2.3 billion illnesses and 1.3 million deaths annually (1). Enteric viruses are responsible for nearly 70% of these cases, with more than 20 distinct viral agents identified to date (2). Among the most common viral causes of diarrhea are members of the Reoviridae, Adenoviridae, *Caliciviridae*, and *Astroviridae* families (3). Since their initial discovery in 1975, astrovirus (AstVs) has been recognized as important etiologic agents of viral gastroenteritis , particularly among infants and young children worldwide, with the burden disproportionately higher in low -and middle-income countries (4, 5).

Astroviruses are non-enveloped, icosahedral viruses measuring approximately28 –30 nm in diameter, and are distinguished by their characteristic star-like appearance. They belong to the Astroviridae family and have a single-stranded, positive-sense RNA genome of about 6.8 kb in length, which is organized into three open reading frames (ORFs). The ORF1a and ORF1b encode non-structural proteins, while ORF2 encodes capsid proteins (4). The *Astroviridae* family includes two genera: *Mamastrovirus* and *Avastrovirus*. Human infections are primarily associated with *Mamastrovirus* species, particularly MAstV-1, MAstV-6, MAstV-8, and MAstV-9. The classic HAstVs (HAstV-1 to HAstV-8) belong to MAstV-1, while recently identified novel strains include HAstV-MLB (MAstV-6), HAstV-VA2/4 and HMO-A (MAstV-8), and HAstV-VA1/3, HMO-B/C, and PS (MAstV-9)(6). While several studies from East African countries have reported the presence of HAstVs among under-five children, most have lacked in-depth genetic characterization. Nevertheless, both classic and novel astrovirus types have been detected in the region. Six of the eight classic HAstV types, excluding HAstV-6 and HAstV-7, have been reported in the region. In Ethiopia, HAstV positivity ranged from 3.6% to 5.3%, with genotypes HAstV-1, 2, 3, and 8 detected (7, 8). In Kenya, positivity rates varied between 2.8% and 9.7% across multiple studies, with both classic and non-classic genotypes such

as MLB and VA strains identified (9, 10-12). Malawi (1.9%), Sudan (3%), Madagascar (2.1%), and Tanzania (0.4%) also reported HAstV cases (113-16), with Malawi and Madagascar identifying multiple genotypes including HAstV-1 to 5 and HAstV-8 (15, 16). Detection methods varied slightly (RT-PCR, ELISA, EIA).

Astroviruses are primarily associated with gastroenteritis; however, growing evidence also indicates their capacity to cause extra-intestinal infections, including meningitis and encephalitis (5, 17). These severe manifestations are often linked to novel astrovirus strains. In Tanzania, MLB1 and MLB2 strains were detected among febrile children, while the AV1 was identified in a nasopharyngeal sample from another febrile child (18, 19). Although HAstVs predominantly affect children (4), they have also been detected in the elderly, immunocompromised individuals, and even healthy adults (20). In immunocompromised patients, infections tend to be more severe and may involve the central nervous system (20, 5). In tropical climate settings, astrovirus infection often follow seasonal patterns, with increased incidence during the rainy season, possibly due to increased viral stability in cool, moist environments and greater indoor crowding. Outbreaks have been documented in high-density communal environments such as schools, daycare centers, military facilities, and swimming pools (6). Transmission of HAstVs is mainly fecal-oral, as confirmed by volunteer studies (21), although the role of other transmission routs warrants further investigation.

Despite the recognized global burden of astrovirus-related gastroenteritis, particularly in young children, research in East Africa remains limited in both population level coverage and depth. Most available studies are confined to under-five children, with little attention to infections in adults, elderly, or immunocompromised populations. Comprehensive genomic based evidence is lacking, with limited data on genotype diversity, recombination potential, and the emergence of novel strains. Additionally, extra-intestinal manifestations, potential environmental reservoirs, zoonotic transmission, and seasonal patterns are underexplored in the regional context. To address these knowledge gaps, expanded active surveillance and molecular studies are urgently needed. Further research should include diverse age groups, clinical presentation, and comprehensive one health approach studies to understand of the transmission dynamics and detail virological properties. F Furthermore, integration of astrovirus monitoring into public health surveillance programs, alongside investment in vaccine and antiviral development, will be crucial for reducing the disease burden and preventing future outbreaks.

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Gene Mutations Conferring Second-Line Drug Resistance in Tuberculosis in Amhara Region: Eight-year Retrospective Study

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Abstract

Background: *Tuberculosis (TB) is an infectious disease that can affect various organs, though it primarily targets the lungs. The global emergence of highly drug-resistant TB strains has significantly undermined treatment and control efforts. Resistance in Mycobacterium tuberculosis is mainly attributed to spontaneous mutations in chromosomal genes. However, data on specific gene mutations associated with second-line drug resistance remain limited in the Amhara Region of Ethiopia.*

Objective: *This study aimed to assess the gene mutations associated with second-line drug-resistant tuberculosis among patients in the Amhara Region, Northwest Ethiopia.*

Method: *A retrospective study was conducted from January 1, 2016, to January 30, 2023. Drug resistance-associated gene mutations were identified using the Genotype MTBDRsl line probe assay. Data were analyzed using SPSS version 26 statistical software. Chi-square test were applied to examine associations between gene mutations and socio-demographic characteristics, with a significance level set at $p < 0.05$.*

Result: *A total of 308 presumptive multidrug-resistant TB (MDR-TB) patients were tested for second-line drug susceptibility. Of these, 165 (53.6%) were male, and the majority (n = 177, 57.5%) were aged 25 - 44 years. HIV co-infection was observed in 41 (13.3 %) patients. Fluoroquinolone (FLQ) resistance due to gyrA mutation at position A90V was identified in 1 (0.3%) isolate. Resistance to second-line injectable drug was observed in 10 (3.2%) isolates, indicated by missing wild-type (WT) bands or mutations in the rrs gene. Among these, 2 (0.6%) had rrs mutations at position A1401G, and 1 (0.3%) at position G1484T. HIV status was significantly associated with FLQ-resistant TB ($\chi^2 = 5.42$, $p = 0.02$), and the year of testing was significantly related to the prevalence of resistance to second-line injectable drugs ($\chi^2 = 13.71$, $p= 0.05$).*

Conclusion: *This study highlights the presence and distribution of gyrA and rrs gene mutations associated with second-line drug resistance in M. tuberculosis isolates from the Amhara Region. The significant association between HIV status and FLQ-resistance underscores the need for integrated TB-HIV management. Routine molecular testing for resistance-conferring mutations is recommended prior to initiating fluoroquinolones and second-line injectable drugs in MDR-TB patients.*

Introduction

The genus Mycobacterium comprises many species, which are typically grouped into three categories: Mycobacterium leprae, the *Mycobacterium tuberculosis* complex (MTBC), and *non-tuberculosis mycobacteria* (NTM)(1). *Mycobacterium tuberculosis (M. tuberculosis)* is a non-motile, non-spore-forming, acid-fast bacterium and a causative agent of tuberculosis (TB)(2).

Tuberculosis is an infectious disease that can affect various parts of the body, although it primarily targets the lungs. Tuberculosis (TB*)* is an infectious disease affecting all parts of the body; primarily affecting the lungs, which in the majority of cases is caused by infection with the intracellular bacterial agent, *M. tuberculosis*(3). Among all bacterial infections, it is the primary cause of human mortality and morbidity worldwide(4).

According to the World Health Organization (WHO), the 2018 report found a high TB burden, particularly in sub-Saharan African countries. Ethiopia was identified as one of the 30 highest TB-burdened countries, with TB remaining one of Ethiopia's leading causes of mortality(5). Once again, Ethiopia is included on the global list of high-burden countries for TB and HIV-associated TB in the period 2021–2025 (6).

Multi-drug resistance tuberculosis (isoniazid and rifampicin resistant TB)(MDR-TB) has caused challenges to global TB control, and it remains a public health crisis and a health security threat (6, 7).

The high prevalence of MDR-TB has hindered the ability of sub-Saharan region, including Ethiopia to control TB effectively(8). Dreadfully, the global rise of extensively drug-resistant tuberculosis (resistant to isoniazid and rifampicin, plus any fluoroquinolone and at least one additional Group A drug (bedaquiline or linezolid)) (XDR TB) has deterred efforts in the treatment and control of TB (9). Extensively drug -resistant tuberculosis is caused by *M.tuberculosis,* which is resistant to any fluoroquinolone and at least one additional Group A drug (bedaquiline or linezolid(10). Extensively drug -resistant tuberculosis is tremendously difficult and expensive to treat and has a very high mortality(11).

The mechanism of drug resistance in *M. tuberculosis* primarily arises from spontaneous mutations in its chromosomal genes (12, 13). Resistant MTB to fluoroquinolones (FQs) exhibit mutations within the quinolone resistance-determining region of the gyrA and gyrB genes (14-16)), with most alterations occurring in a short region*(17).* A significant number of mutations identified in the quinolone resistance-determining region of the *gyrA* gene have been associated with resistance to FQs (18). In addition, the rrs and eis genes are involved in second-line injectable drug resistance (associated with resistance to amikacin, kanamycin, and capreomycin) in *MTB*(19, 20). A global study indicated that resistance to second-line injectable drugs was linked to mutations in the *rrs* gene(21), while polymerizations of the *rrs* gene have various adverse effects on treatment success and antibiotic resistance(22).

The WHO claims that detection of drug resistance requires bacteriological confirmation using rapid molecular tests, culture, and sequencing technology before linking the MDR-TB patients to the TIC(6). However, very little data is documented in other parts of Ethiopia about the burden of *gene* mutation on tuberculosis conferring second drug resistance. Therefore, we believe that the Amhara community's lifestyle, culture, and geographic location are different from those of Ethiopian communities, and therefore, the risk of developing drug-resistant TB and gene mutation on MDR-TB may differ. In addition, the burden of *gene* mutation on tuberculosis and the drug resistance profile of *M. tuberculosis* remain largely underexplored in our study area. Therefore, the present study was conducted to detect the frequency of mutant genes conferring second-line drug resistance and its associated factors among MDR TB patients by using the version two -line probe assay MTBDRsl PCR technique.

Method

Study design and setting

A descriptive retrospective study was conducted between January 2016 and January 2023 among MDR-TB patients in the Amhara National Regional State Public Health Institute (APHI), Amhara Region, North West Ethiopia. Amhara National Regional State has 100 hospitals and 917 health centers serving over 25.5 million people. It has 18 MDR-TB treatment initiation centers (TIC), and two facilities offering

the TB culture test (APHI and University of Gondar Comprehensive Specialized Hospital). The APHI is an institute having a BSL-3 TB laboratory, fully furnished to manipulate TB cultures and anti-TB Drug susceptibility tests. It receives samples for TB culture from 11 MDR-TB TICs through an integrated specimen referral system. The integrated specimen referral system covers multiple disease programs, including HIV and TB. Specimen referral services are provided by the Ethiopian Postal Service Enterprise (EPSE) for all health service providers, including peripheral facilities. The postal specimen referral service is a schedule- and phone call-based service that uses vehicles and motorbikes to transport specimens to testing facilities.

Study population

The source population was all MDR-TB patients from the Amhara region whose sputum sample had culture-positive results in APHI

Data collection and laboratory protocols

The socio-demographic characteristics and risk factors were collected from the registration book. Three hundred eighty-eight second-line LPA results were collected from the result reading and reporting template.

Sputum Specimen Collection and Culture Processing for *M. tuberculosis*

All samples were transported to APHI under a cold chain based on the Ethiopian National TB specimen transport systems. Upon receipt, samples were inspected for the minimum sample acceptance criteria(Sample volume greater than 2ml, if the sample was received less than 5 days of collection, if the cold chain was 2-8 ^{o}C, correctly labeled, or leak-proof container) before culture processing (23). Standard procedures were followed during the mycobacterial culturing steps(24). The growth of MTB colonies on LJ culture media was inspected weekly for up to 8 weeks, and the growth was confirmed by Ziehl-Neelsen (ZN) smear staining(25). "Capilia TB-Neo (Tauns Laboratories, Japan)" was utilized to confirm whether the isolates was MTBC or not(26).

GenoType MTBDRsl Assay

M. tuberculosis colonies were scraped from solid media with sufficient growth and added into screw cupped tube containing lysis buffer. The Life Science GenoType MTBDRsl VER 2.0 kit was used to extract the DNA of *M.tuberculosis*(27). The protocol for cultured specimens was selected in multiplex PCR to amplify genes liable for drug resistance such as gyrA, gyrB, and *rrs* genes(27).

The appropriate program was selected for the hybridization in Twin-Cubator. All steps in denaturation, hybridization, conjugation, rinsing, and addition of substrate solution were followed accordingly to see visible bands on the DNA strips. The visible bands on the DNA strip were interpreted according to the instructions given by the HAIN Life Science user manual (27, 28).

Laboratory quality control

The internal quality control (IQC) was tested during each run performed. In each of the LPA tests (MTBDR*sl* VER2.0), sterile water was utilized as the negative control, while the universal reference H37Rv strain, which is sensitive to all anti -TB medicines, was utilized as a positive control. The test reagents and consumables used for testing clinical samples also had records of LOT-to-LOT testing to ascertain performance.

Data analysis and interpretation

Data were checked for completeness, cleaned in Microsoft Excel 2016, and entered into SPSS version 26 software. The results were presented through tables and charts. Means and standard deviations were calculated for continuous variables while the Pearson correlation coefficient was calculated to check the statistical association between the dependent and independent variables using the Chi-square test. P-values less than 0.05 were considered statistically significant.

Result

A total of 308 presumptive MDR-TB patients were tested for second-line drug susceptibility using GenoType MTBDRsl during the study period from 2016 to 2023. Out of this 165 (53.6%) were males. The majority 177(57.5%), of the patients belonged to the 25-44 years age group and 41(13.3 %) were HIV positive. Most 238(77.3%) were new in their TB registration group. About 123 (39.9%) patients were negative for smear microscopy during the LPA test (Table 1).

Table 1: Socio-demographic and clinical information of DR TB Patients in APHI, Ethiopia

Variables		Frequency	Percent
Sex	Female	143	46.4
	Male	165	53.6
Age Category	<25	78	25.3
	25-44	177	57.5
	>44	53	17.2
HIV status	Negative	267	86.7
	Positive	41	13.3
Reason for Test	Diagnosis	83	26.9
	Follow up	225	73.1
Patient TB Registration Group	Failure	29	9.4
	LFU	6	1.9
	New	238	77.3
	Relapse	35	11.4
AFB Microscopy Results	Negative	123	39.9
	Scanty	23	7.5
	P+1	83	26.9
	P+2	42	13.6
	P+3	37	12.0
Year of Examination	2016	9	2.9
	2017	14	4.5
	2018	63	20.5
	2019	69	22.4
	2020	61	19.8
	2021	31	10.1
	2022	43	14.0
	2023	18	5.8

Based on the MTBDRsl assay, out of 308 isolates, 1 (0.3%) had a mutation in the *gyrA* gene of *Mycobacterium tuberculosis*, where the amino acid alanine (A) at position 90 is replaced by valine (V).

Additionally, 10 (3.2%) strains either lacked their WT band or had mutations in the *rrs* genes. Four (1.3%) isolates had missed their wild-type 1 genes, while 3(1%) of isolates had missed their wild-type 2 genes in the *rrs* gene. Two (0.6%) of the isolates had a change from adenine (A) to guanine (G) at nucleotide position 1401, and 1(0.3%) of the isolates had a change from guanine (G) to thymine (T) at nucleotide position 1484 within the *rrs* gene (Table 2).

The chi-square test was used to assess associated factors for the presence of fluoroquinolone-resistant TB and second-line injectable drugs. Patient HIV status was associated with the presence of FLQ-resistant Mtb (X2 =5.42, p-value =0.02) (Table 3). The years of examination were significantly related to the prevalence of second-line injectable drugs (X2=13.71, p-value =0.05) (Table 4).

Table 2: Frequency of gene mutation of *M. tuberculosis* associated with second-line anti-TB drug resistance in 2016 to 2023 (n=308)

Anti-TB determining regions	Type of Gene	Band	Mutant probe	Strains with a missed or mutant gene	
				Number	Percent
Fluoroquinolones resistance-determining regions	*gyrA*	*gyrA* WT1	85–93/92–96	0	0
		gyrA WT2	85–93/92–96	1	0.3
		gyrA WT3	85–93/92–96	0	0
		gyrA MUT1	A90V	1	0.3
		gyrA MUT2	S91P	0	0
		gyrA MUT3A	D94A	0	0
		gyrA MUT3B	D94N/Y	0	0
		gyrA MUT3C	D94G	0	0
		gyrA MUT3D	D94H	0	0
	gyrB	*gyrB* WT	536–541	0	0
		gyrB MUT1	N538D	0	0
		gyrB MUT2	E540V	0	0
Second-line injectable drug resistance-determining regions	*rrs*	*rrs* WT1	1400/1484	4	1.3
		rrs WT2	1400/1484	3	1
		rrs MUT1	A1401G	2	0.6
		rrs MUT2	G1484T	1	0.3
	eis	*eis* WT1	G-37 to -2A	0	0
		eis WT2	G-37 to -2A	0	0
		eis WT3	G-37 to -2A	0	0
		eis MUT1	C-14T	0	0

Table 3: Chi-square test of factors associated with FLQ resistance TB (N=308).

Variables		Frequency No (%)	*FLQ result*		Chi-square (X2)	*p*-value
			S	***R***		
Sex	Female	143(46.4)	138(96.5)	5(3.5)	1.83	0.18
	Male	165(53.6)	163(98.8)	2(1.2)		
Age Category	<25	78(25.3)	77(98.7)	1(1.3)	0.67	0.72
	25-44	177(57.5)	172(97.2)	5(2.8)		
	>44	53(17.2)	52(98.1)	1(1.9)		
HIV status	Negative	267(86.7)	263(98.5)	4(1.5)	**5.42**	**0.02**
	Positive	41(13.3)	38(92.7)	3(7.3)		
Reason for Test	Diagnosis	83(26.9)	82(98.8)	1(1.2)	0.66	0.42
	Follow up	225(73.1)	219(97.3)	6(2.7)		
Patient Registration Group	Failure	29(9.4)	27(93.1)	2(6.9)	3.74	0.29
	LFU	6(1.9)	6(100)	0(0)		
	New	238(77.3)	233(97.9)	5(2.1)		
	Relapse	35(11.4)	35(100)	0(0)		
AFB Microscopy Results	Negative	123(39.9)	122(99.2)	1(0.8)	4.71	0.32
	Scanty	23(7.5)	23(100)	0(0)		
	P+1	83(26.9)	81(97.6)	2(2.4)		
	P+2	42(13.6)	40(95.2)	2(4.8		
	P+3	37(12)	35(94.6)	2(5.4)		
Year of Examination	2016	9(2.9)	8(88.9)	1(11.1)	9.92	0.19
	2017	14(4.5)	13(92.9)	1(7.1)		
	2018	63(20.5)	60(95.2)	3(4.8)		
	2019	69(22.4)	68(98.6)	1(1.4)		
	2020	61(19.8)	61(100)	0(0)		
	2021	31(10.1)	30(96.8)	1(3.2)		
	2022	43(14.0)	43(100)	0(0)		
	2023	18(5.8)	18(100)	0(0)		

Table 4: Chi-square analysis of Factors associated with CAP, KAN, resistance TB (N=308).

Variables		Frequency No (%)	*rrs*		Chi-square (X2)	*p*-value
			S	***R***		
Sex	Female	143(46.4)	138(96.5)	5(3.5)	0.05	0.82
	Male	165(53.6)	160(97)	5(3)		
Age Category	<25	78(25.3)	77(98.7)	1(1.3)	2.42	0.30
	25-44	177(57.5)	169(95.5)	8(4.5)		
	>44	53(17.2)	52(98.1)	1(1.9)		
HIV status	Negative	267(86.7)	258(96.6)	9(3.4)	0.10	0.75
	Positive	41(13.3)	40(97.6)	1(2.4)		
Reason for Test	Diagnosis	83(26.9)	79(95.2)	4(4.8)	0.83	0.36
	Follow up	225(73.1)	219(97.3)	6(2.7)		
Patient Registration Group	Failure	29(9.4)	27(93.1)	2(6.9)	1.42	0.7
	LFU	6(1.9)	6(100)	0(0)		
	New	238(77.3)	231(97.1)	7(2.9)		
	Relapse	35(11.4)	34(97.1)	1(2.9)		
AFB Microscopy Results	Negative	123(39.9)	119(96.7)	4(3.3)	2.45	0.65
	Scanty	23(7.5)	23(100)	0(0)		
	P+1	83(26.9)	81(97.6)	2(2.4)		
	P+2	42(13.6)	40(95.2)	2(4.8)		
	P+3	37(12)	35(94.6)	2(5.4)		
Year of Examination	2016	9(2.9)	9(100)	0(0)	**13.71**	**0.05**
	2017	14(4.5)	14(100)	0(0)		
	2018	63(20.5)	59(93.7)	4(6.3)		
	2019	69(22.4)	67(97.1)	2(2.9)		
	2020	61(19.8)	61(100)	0(0)		
	2021	31(10.1)	31(100)	0(0)		
	2022	43(14.0)	39(90.7)	4(9.3)		
	2023	18(5.8)	18(100)	0(0)		

Discussion

For TB to be effectively treated and controlled, gene changes on MTB that confer drug resistance must be regularly surveyed and continuously monitored. To develop targeted TB management methods in the region, it is essential to comprehend local gene changes in TB that result in treatment failure. Therefore, this eight-year retrospective data was collected to assess fluoroquinolone and second-line injectable drug resistance-determining genes in *M. tuberculosis*.

In this study, among the 308 isolates, the *gyrA* mutation indicated that 1(0.3%) was resistant to fluoroquinolones, which is lower than the frequency of *gyrA* gene mutation in South Africa, 1.3% (29), and Morocco 2.22% (30). Even a very high prevalence of *gyrA* gene mutations was documented in studies in China, with 62% (31), Russia 94.7% (32), Uzbekistan 89% (33), and India, 39% (34). Fluoroquinolones resistance-determining regions, such as codon D94G, A90V, and S91P of *gyr*A in fluoroquinolone-resistant TB were frequently reported in several studies (35-40). In our results, the FLQ resistance gene in *gyrA* was positioned at A90V, which is in agreement with a laboratory-based surveillance study in Ethiopia(41), Central, Southeastern, and Eastern Ethiopia (36), and a report from Morocco(42). These differences may be due to variations in MTB stain, study period, geography, study population, study design, and methods.

Our study found no isolates harbored mutations in the *gyrB* and eis genes. Likewise, no mutations were detected at *gyrB* in a previous study in Northwest Ethiopia (43) and Central, Southeastern, and Eastern Ethiopia(36). Consistent with our findings, there was no *gyrB* mutation in tuberculosis strains in the Moroccan study(44). In contrast, the frequency of *gyrB* gene mutation was 4% (45) in France and 3.3% in South Africa (46), 7.7%) in Germany (33), 7.7% in Russia (47), and 5.9% in China(48). These variations in *gyrA* and *gyrB* gene mutations may be due to the higher MDR TB strain in China, Russia, and India, and higher pre-XDR and XDR TB circulating in their populations. Based on the WHO global report, these countries have the largest numbers of XDR TB cases globally (5, 6), which supports the above statement. Additionally, this discrepancy might be due to the significant geographical variation of TB lineage distribution, as studies showed that the Central African and East African-Indian lineages were confined to East Africa, while the East Asian lineage was predominantly found in Southern Africa (49, 50). Furthermore, it was revealed that Mycobacterium tuberculosis Sub-Lineage 4.2.2/SIT149 was the predominant Drug-Resistant Clade in our study area, which could be the reason for the discrepancies in the outcomes(51).

Scholars showed that mutations in several genetic loci of Mtb strains had been implicated in the development of resistance to second-line injectable anti-TB drugs, such as resistance to KAN, AMK, and CAP (52, 53). Based on our results, 10 (3.2) % of strains either missed their WT band or mutated at the rrs genes, indicating second-line injectable drug resistance, which was similar to the studies in Myanmar (3.3%)(54). Disparity, the prevalence of *rrs* gene mutation was lower in Kenya (1.5%) (55) and no mutation on the rrs gene in the Moroccan study(56).

On the other hand, the frequency of rrs gene mutation in our study is lower than the frequency of *rrs* gene mutation in China (88.5%) (57), (94.9%) in South Africa (58), (92%) in Latvian study(59), (53.33%) in Indian (60), 25% in Atlanta(61), (44.1%) in Thailand(62), and (89.65%) strains in India(63). These differences may be due to the variation in the prevalence of pre-XDR and XDR TB among the countries, variations of tuberculosis strain in the study areas, the study period, and the sample size. Moreover, the method used to assess the *rrs* gene mutation may cause a difference in the prevalence of *rrs* gene mutation. For instance, Kumari, et al. (63) used the primers for multiplex allele-specific PCR assays, designed to identify *gyrA* D94G and *rrs*A1401G mutations, and the agarose gel images of PCR products were applied to detect gene mutation. In the present study; commercially available Genotype MTBDRsl kits were used to interpret any gene mutation. The researcher also stated that the accuracy of the result depends largely on the strength of the association between a specific mutation and the phenotypic resistance of the isolates(64), which supports our explanation for the discrepancy.

In the present study, 2 (0.6%) Mycobacterial isolates had the rrs mutation at position A1401G, and 1 (0.3%) at the G1484T. Similarly, the A1401G mutation was reported as the most frequent codon in Ethiopia(41) and a systematic review also noted that resistance to second-line injectable drugs is mostly associated with *rrs* A1401G mutation(64).

Scholars have found that diabetic mellitus, age, previous treatment history, and HIV are among the risk factors for anti-tuberculosis resistance (65-68). However, in our data, we observed that patient HIV status was the only factor associated with the presence of second-line injectable drug-resistant TB (X2 =5.42, p-value =0.02). Similarly, the positive association between HIV and anti-tuberculosis resistance was observed in a study(69). Inconsistent with our study, HIV serostatus was not associated with fluoroquinolone resistance in the United States population(70). Eldholm, V., et al. conclude in their study that HIV co-infection does not significantly affect the mutation rate of Mtb within patients and is not associated with the emergence of resistance(71). This variation may be due to differences in the assessment method, the study population, the prevalence of HIV in the community, and the study periods.

Conclusion

In conclusion, the present study shows the distribution of *gyrA* and *rrs* gene mutations in this study area. Moreover, patient HIV status was associated with the presence of FLQ-resistant TB. Thus, MDR-TB patients in the study area should be monitored for gene mutation before the initiation of fluoroquinolone and second-line injectable drugs.

Data availability statement: All the data sets analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical consideration: The study was approved by the Ethical Review Committee (ERC) of the Health Research Development Directorate of Amhara regional state with reference number NoH/R/T/T/D/07/88.

Participant information was treated confidentially, and specimens collected were used solely for the study's intended purposes. All procedures in this study were conducted by the amended Declaration of Helsinki(72).

Author contributions: GB, HG, and YG contributed to conceiving the research idea, data collection, and data analysis. GB, MG, AT, TM, TB and BB contributed to the conception of the research idea, method rationalization, data analysis, interpretation of results, evaluation of scientific content, and manuscript preparation. GB, YG, MG, AA, TM, AT, and BB were also involved in reviewing and editing the manuscript. All authors had read and approved the final manuscript for submission.

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Abbreviations: APHI: Amhara Public Health Institute, CLSI: Clinical and Laboratory Standards Institute, FLQ: Fluoroquinolone, LPA: Line Probe Assay, MDR-TB: Multi-Drug Resistance Tuberculosis, XDR-TB: Extensively Drug Resistance Tuberculosis, WT: Wild Type

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Evaluation of Crystal® VC Rapid Diagnostic Test kit to detect *Vibrio cholerae* from fecal samples in Ethiopia

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Abstract

Background: *The Crystal® VC Rapid Diagnostic Test (RDT) has been shown to be a sensitive, rapid, cost-effective, and time-efficient tool for detecting V. cholerae. Although this test has advantages in terms of sensitivity and specificity, there are no reports on how well it performs across different regions and during various seasons in Ethiopia to assess its robustness.*

Objective: *The study aimed to evaluate the diagnostic performance of Crystal® VC RDT rapid Diagnostic Test to detect V. cholerae from fecal samples in Ethiopia.*

Method: *A cross-sectional study was conducted from October 2022 to February 2024 at cholera outbreak sites across Ethiopia. A total of 361 fecal samples were collected and tested concurrently using Crystal® VC RDT and standard culture methods. Sensitivity, specificity and positive and negative predictive value of the Crystal® VC RDT were calculated using culture as the gold standard. Agreement between the two diagnostic methods was assessed using Cohen's Kappa statistic. Data analysis was performed using SPSS version 25 and MedCalc computer software.* ***Results:*** *Of the 361 fecal samples analyzed, 123 (34%) were confirmed positive for Vibrio cholerae by culture. The Crystal® VC RDT demonstrated a sensitivity of 98.4% (95% CI: 94.3% –99.8%) and a specificity of 52.1% (95% CI: 45.6%–58.6%).The PPV was 51.5% (95% CI: 48.1%–54.8%) and the NPV was 98.4% (95% CI: 94.0%–99.6%). The test showed a moderate agreement with culture (kappa =0.679).*

Conclusion: *Crystal® VC RDT demonstrate high sensitivity and excellent NPV, making it a valuable tool for early detection and rapid response during cholera outbreaks. Despite lower specificity, the test's speed and ease use support its utility in field settings and epidemiological surveillance in resource-limited areas.*

Introduction

Cholera is an acute bacterial gastrointestinal non-invasive disease that affects many low- and middle-income countries (1). Individuals diagnosed with cholera exhibit acute watery diarrhoea, experiencing three or more episodes within a 24-hour period (2). Cholera is caused by *Vibrio cholerae* (*V. cholerae*) which is a Gram-negative bacteria (3). Cholera outbreak is a major public health emergency disease, it results high socioeconomic disruption, morbidity and mortality across the world (4, 5). Currently, there are an estimate of 1.3-4.0 million cholera cases and 21,000-143,000 cholera annual deaths in the world. Cholera remains a global threat to public health which is also an indicator of inequity and lack of social development (6, 7). The global burden of cholera is high in all age groups. However, the incidence and mortality increased in children under five years old (8).

In Ethiopia, cholera impacts 70 million people each year, with an estimated 275,221 cases and 10,458 deaths annually (9). As Office for the Coordination of Humanitarian Affairs (OCHA) report, about 29,800 cholera cases and more than 400 deaths were reported across 10 regions of Ethiopia between January 2023 and January 2024. According to Access Capacities Project (ACAPS) thematic cholera report in Ethiopia, conducted on 18th January 2024, the highest number of cases were reported in Oromia National Regional State, Amhara National Regional State, and Somali National Regional State, respectively (10).

The identification of the causative agents of epidemic cholera requires microbiological diagnostic methods, such as culture, PCR, and RDT (11). Culture techniques can be utilized to grow, isolate, and characterize *V. cholerae* (12). Culture is gold standard method for detecting *V. cholerae*. This is then followed by biochemical identification and serotyping using both polyclonal and monoclonal antibodies (13). However, PCR offers greater accuracy and sensitivity for *V. cholerae* detection and is increasingly adopted in diagnostic laboratories for timely and reliable results (14). In resource limited settings, culture and PCR methods for detecting *V. cholerae* are often impractical due to their lengthy processing times (at least 18 hours for culture), the need for highly trained personnel, high costs, and the requirement for specialized laboratory infrastructure (15). Nowadays, Crystal® VC RDT has been developed and is accessible for detecting the causative agents of cholera from clinical samples (16). The new version of Crystal® VC RDT kit was manufactured by Arkray Healthcare Pvt. ltd, at Gujarat, India (17). Crystal® VC RDT was designed to detect *V. cholerae* O1 and O139 in fecal sample using a rapid visual immunochromatographic assay, and has sensitivity range 88-100% and specificity 61-87.3% (18, 19). Crystal® VC RDT is easy to use and can be used as a portable device from one location to another (20). In addition, Crystal® VC RDT is utilized at point-of-care facilities to enhance decision-making in the timely management of cholera outbreaks and to investigate the epidemiology of the disease, particularly during surveillance activities. This helps to minimize the spread of outbreaks and reduce mortality (21). As far as we know, there are no reports on how well the recently introduced Crystal® VC RDT test performed in Ethiopia. Therefore, this study aimed to evaluate the performance of Crystal® VC RDT for detecting *V. cholerae* from diarrhoea samples in Ethiopia.

Method

Study area, design and participants

A cross-sectional study was carried out from October 2022 to January 2024 at outbreak sites in the three regional states in Ethiopia. Fecal samples were collected from each participants suspected of cholera and attending treatment at the different outbreak sites in Oromia National Regional State (Bale zone, Guji zone, west Arsi and Madda Walabu), Amhara National Regional State (West Gondar zone, Bahir Dar Zuria and Awi zone) and Addis Ababa City Administration (Kolfe Qeranio sub-city).

Sample size: The sample size was determined using the single population proportion formula.

$$n=Z^2 * p(1-p)/E^2$$

p= population proportion=50%

z= z-score=1.96

E = margin of error=5%

n= study sample size

$$n= (1.96)^2 * 0.5(0.5)/(0.05)^2 = 384$$

First a total of 384 individuals suspected of cholera were enrolled. Twenty-three participants were excluded from the study due to mislabeling or delays in collection of their fecal samples. The final sample size was 361. Of these, the majority of the study participants were collected from Oromia Na-

tional Regional State (223), from Amhara National Regional State (73), from Addis Ababa City Administration (65). The study participants were selected using convenient sampling technique.

Inclusion and exclusion criteria

Participants who were admitted to the cholera treatment center (CTC) at each outbreak site due to acute watery diarrhoea were included. However, participants who had been on antimicrobial treatment for the past two weeks before sample collection were excluded from this study.

Fecal sample collection, storage and transportation

According to the national cholera sample collection protocol, two aliquot of fecal samples were collected from each patient suspected of cholera disease (22). The fecal samples were collected by using wide mouthed, leak proof, clean, and dry container. One of the aliquot of the fecal sample was used for detection of *V. cholerae* with Crystal® VC RDT (Arkray Healthcare Pvt. ltd, at Gujarat, India) at the CTC and the other aliquot of the fecal sample was used for culture to isolate *V. cholerae* at the microbiology diagnostic laboratories.

Crystal® VC Rapid Diagnostic Test

Aliquot of fecal samples were immediately tested using the Crystal® VC RDT to detect the presence of *V. cholerae* O1 and/or O139 at the CTC. Briefly, 200µl of fresh watery fecal samples were taken by a pasture pipette and dispensed directly to the well of the Crystal® VC RDT test kit. The preparation was allowed to stand for 15 - 30 minute at room temperature until the test and the control line are visible and removed after 30 minutes. The Crystal® VC RDT results were observed and the results determined as positive or negative by two medical laboratory science professionals. A positive result appears as two or three pink lines on the kit, the one being the control band and the other line being the band specific to either serogroup O1, or serogroup O139 or both serogroups. The results were recorded and interpreted according to the manufacturer's protocol. If the control line did not appear visible regardless of the test lines, the test was considered invalid and repeated once (21).

Detection of *V. cholerae* using Culture

The second aliquot of fecal samples were placed into Kari Blair transport media and kept cold using an ice pack and then transported to the nearby microbiology laboratory available sites to Shashemene General Hospital, Armauer Hansen Research Institute and Amhara Public Health Institute for sample processing and laboratory analysis. Fecal samples were inoculated onto Blood Agar (BAP, Oxoid), MacConkey Agar (MAC, Oxoid), and Thiosulfate Citrate Bile Salt Sucrose (TCBS, Oxoid) agar, and then incubated at 37°C for 24 hours. Identification of *V. cholerae* was performed using a series of biochemical tests, including the Oxidase test, String test, Motility test, Indole test, Citrate test, Gas production test, H2S production test, and Urease test, all from Oxoid. Polyvalent and monovalent antisera (manufactured by Deben Diagnostics Ltd, USA) were utilized for agglutination tests to identify and differentiate *V. cholerae* serogroups and serotypes. The culture method was performed independently of the Crystal® VC RDT results to ensure unbiased outcomes.

Quality control

Data collected daily was recorded and compiled. A laboratory protocol was prepared and strictly followed. The principal investigator was responsible for monitoring all steps of data collection and recording. The reagents were checked for expiry date and appropriate storage temperature and humidity. In parallel, both positive and negative controls were included. *Vibrio cholerae* reference strains; N16961 or C6706 (O1 El Tor) and MO45 (O139) were used as controls based on the combination of conventional biotyping methods. Quality assurance was ensured with good practice in preparing and reading.

Data Analysis

All data were coded and checked to detect an error and transferred from a questionnaire to excel, and then to SPSS version 27. The definition conventional culture was used as gold standard and considered as a reference for Crystal® VC RDT. The sensitivity, specificity, predictive value, prevalence and accuracy were analyzed by SPSS version 25, and MedCalc statistical software. The kappa (k) statistics and ROC curve were calculated by SPSS and used to compare the agreement between the RDT result and the Gold standard culture result. The interpretation of the agreement was as follows no agreement if k value < 0, poor agreement if k value = 0 – 0.2, fair agreement if k value = 0.21 – 0.4, moderate agreement if k value= 0.41-0.6, substantial agreement if k value= 0.61- 0.8 and excellent agreement if k value > 0.8 (23).

Ethical considerations

Ethical clearance was obtained from the University of Gondar Institutional Review Board (IRB) (R. No. *VP/*

RTT/05/20/2022). Written informed consent was obtained from participants after explaining the purpose and objective of the study. In addition, formal written consent was obtained from the parent/guardian. Participants had a full right to continue or withdraw from the study. All information was kept confidential by assigning code and assessed only by the principal investigator and supervisors. The laboratory results were communicated with concerned stakeholders and participants. If the fecal sample was positive either one or both methods for *V. cholerae*, we communicated with concerned health professionals, and treated patients according to WHO cholera outbreak management guidelines.

Result

A total of 361 participates suspected of cholera were included and fecal samples were collected. The data indicated that 54.9% of the study participants were female, and 52.8% participants were living in rural areas. The average age of participants was 23 years, with a range covering from 1 to 80 years. Nearly half (47.2%) of the participants were aged between 6 and 18 years **(Table 1).**

Table 1: Demographic characteristics of participants involved in cholera outbreak study in Ethiopia.

Variables	Category	Frequency	Crystal® VC RDT positive (%)	Culture positive (%)
Age	1-5	85	46 (54.1)	10 (11.8)
	6-18	166	118 (70.1)	65 (39.2)
	≥19	110	71 (64.5)	48 (43.6)
Sex	Male	164	112 (68.3)	59 (36)
	Female	197	123 (62.4)	64 (32.5)
Region	Oromia	225	125 (55.6)	34 (15.1)
	Amhara	71	67 (94.4)	59 (83.1)
	Addis Ababa	65	43 (66.2)	30 (46.2)
Residence	Urban	172	111 (64.5)	55 (32)
	Rural	189	124 (65.6)	68 (36)
Educational status	Can't read and write	145	88 (60.7)	31 (21.4)
	Elementary	189	129 (68.3)	77 (40.7)
	Secondary	4	4 (100)	4 (100)
	Higher	23	14 (60.9)	11 (47.8)
Marital status	Married	160	113 (70.6)	79 (69.9)
	Unmarried	186	115 (61.8)	38 (20.4)
	Divorced	11	6 (54.5)	5 (45.5)
	Widowed	4	1 (25)	1 (25)
Frequency of diarrhea	1-3	34	24 (70.6)	11 (32.4)
	≥3	327	211 (64.5)	112 (34.3)

Crystal® VC RDT: Crystal *Vibrio Cholerae* Rapid Diagnostic Test

Evaluation of Crystal® VC RDT to detect *Vibrio cholerae* compared with culture

The study showed that (65.1%, n=235) of fecal samples were found positive for *V. cholerae* by using the Crystal® VC RDT, while (34.1%, n=123) of fecal samples were detected positive for *V. cholerae* by using the culture method. On the other hand (34.9%, n=126) of the samples were negative for *V. cholerae* by both methods (Table 2).

In the present study, the Crystal® VC RDT demonstrated the following performance metrics: sensitivity, specificity, positive and negative predictive value were revealed as follows (Table 3).

Table 2: Comparison of the e Crystal® VC RDT and the conventional culture method.

Test methods		Culture (N=361)		Total
		Positive	negative	
Crystal® VC RDT (N=361)	positive	121	114	235
	negative	2	124	126
Total		**123**	**238**	**361**

N: Number

Table 3: Diagnostic performance of the Crystal® VC RDT test compared with the gold standard culture method to detect *V. cholerae* from fecal samples.

Parameters	Performance (%)	95%CI
Sensitivity	98.4	94.3 - 99.8
Specificity	52.1	45.6 – 58.6
Positive predictive value	51.5	48.1 -54.8
Negative predictive value	98.4	94.0- 99.6
Prevalence	34.1	29.3 – 39.2

CI: confidence interval

Using the culture method as gold standard for detection of *V. cholerae*, the kappa agreement of the Crystal® VC RDT was 67.9% (p=0.001).

Receiver operating characteristic curve (ROC) curve analysis

Moreover, the overall accuracy of the Crystal® VC RDT was deemed acceptable with an area under the ROC curve was determined 67.9% (95%CI: 62.8% – 72.7%) (**Figure 1).**

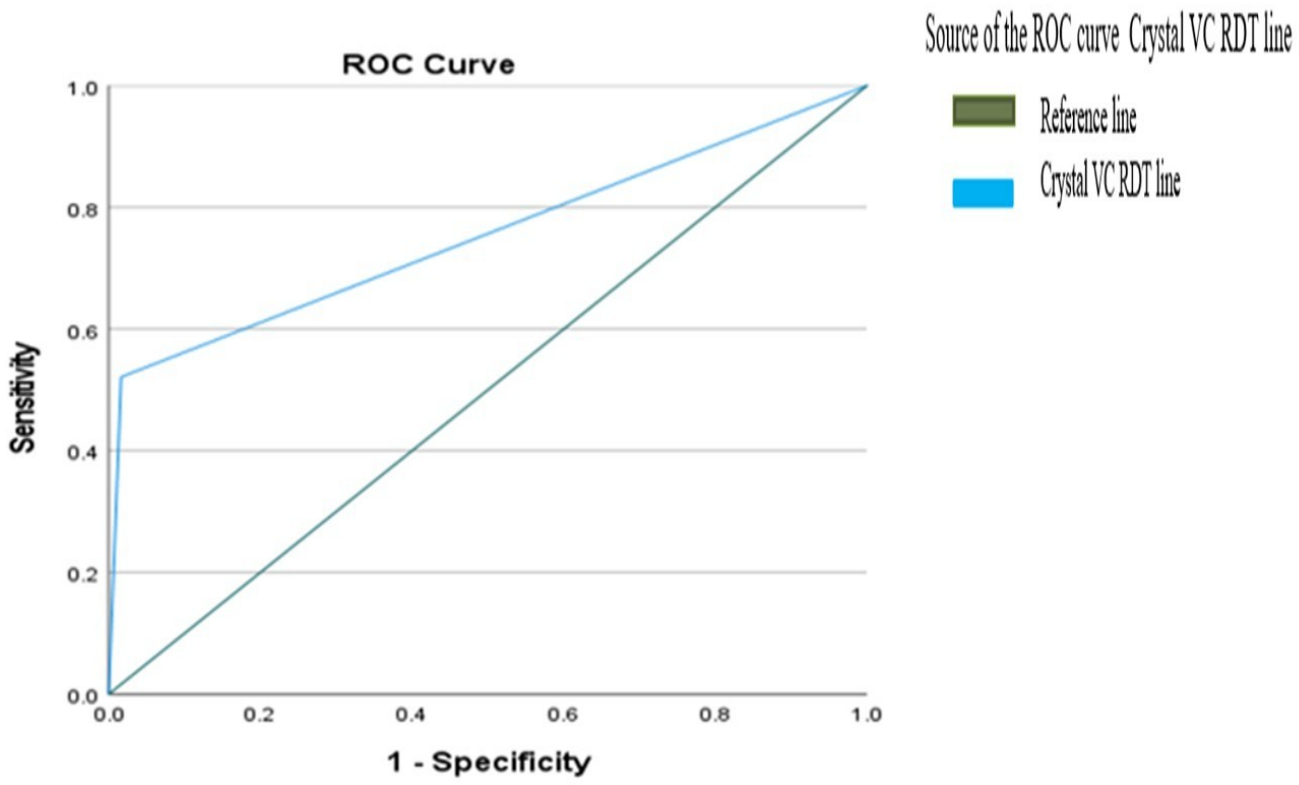

Figure 1. Receiver operating characteristic (ROC) curve for Crystal® VC RDT kit. The X-axis represents false positive rate (1-specificity) and the Y-axis represents true positive rate (sensitivity). The diagonal green line represents random classification (reference line. Thus, the ROC curve is a plot of tests sensitive versus 1-specificity as well. The closer this curve is to the upper left corner, the better the diagnostic significance.

Discussion

Crystal® VC RDT kit has been marketed as an alternative to the conventional culture method, particularly in high cholera outbreak attacked countries having resource-limited laboratory settings (17). The current study assessed the significance, and performance of the Crystal® VC RDT kit in comparison to the conventional culture method, which is considered as the gold standard for *V. cholerae* diagnosis.

In this study, the sensitivity of the Crystal® VC RDT was 98.4% (95%CI; 94.3% - 99.8%). Previously, Chowdhury et al. from India showed exactly a similar report (98.4%) of sensitivity of Crystal® VC RDT compared with gold standard culture (24), and another report from the Democratic Republic of Congo demonstrated lower sensitivity (92.2%) (16), and in Hati, Lower sensitivity (71.2%) and lower positive predictive value (81.3%) were previously reported (25). The present study findings were greater than the previous WHO sensitivity, specificity, positive predictive value, negative predictive value reports of 91.3%, 43.1%, 72.8%, 74.8%, respectively (26). This observed discrepancy may be attributable to several methodological and contextual variables, such as sample quality, differences in the technical proficiency and experience of healthcare personnel, and heterogeneity in the types of biological specimens analyzed. Each of these factors has the potential to influence diagnostic accuracy of the current and previous WHO investigations (27).

The specificity of the Crystal® VC RDT was relatively lower 52.1% (95%CI; 45.6% – 58.6%). The study was comparable with Ley B. et al. specificity report in Zanzibar (49.2%) (19). Data of the present study on the positive and negative predictive values for the Crystal® VC RDT were 51.5% and 98.4%, respectively. This study findings were aligned with the manufacturer's report range for the Crystal® VC RDT, which is 88-100% for sensitivity and 61-87.3% for specificity (17, 24). Collective evidence shows that Crystal® VC RDT is a test with high sensitivity, high negative predictive value, and low specificity, with lower positive predictive values.

In the present study, the Crystal® VC RDT had a moderate Kappa agreement of 67.9% (p=0.001) with the gold standard culture. The kappa agreement of our study was less than the kappa agreement of 98.06% a study conducted in Kenya (28). In addition, Crystal® VC RDT had moderate accuracy under the ROC curve analysis of 67.9% (95%CI= 62.8%–72.7%). Crystal® VC RDT does not require exclusive equipment and facilities which makes it easy and applicable in resource limited settings like Ethiopia. In general, the findings indicate that the Crystal® VC RDT showed moderate agreement with the gold standard culture method, affirming its reliability to use as a *V. cholerae* detection tool during cholera outbreak in resource limited settings and health institutions (21).

Having higher sensitivity to Crystal® VC RDT is very essential, especially during the occurrence of cholera outbreak (29). Because a highly sensitive diagnostic test increases the detection rate of the causative agents of the cholera outbreak (20). This helps to detect *V. cholerae* rapidly among infected patients and will be helpful in providing immediate patient management (25). The use of Crystal® VC RDT is more essential at the point-of-care facilities as it helps to make appropriate decisions in the management of outbreaks or epidemiological surveillance by the public health authorities (30). Crystal® VC RDT is simple easy to use, fast, cheap and can be stored without refrigeration (13, 31). Crystal® VC RDT is used to detect lipopolysaccharide antigens from *V. cholerae* O1 and/or O139 serogroups in fecal samples, which are also present in oral cholera vaccines (32). Hence, Crystal® VC RDT test could be used as a point of care test (POCT) detection tool to *V. cholerae* and used to detect vaccine efficacy within one week after vaccine providing (33).

Furthermore, this Crystal® VC RDT also helps to the Global Task Force on Cholera Control's (GTFCC) roadmap/ program to End Cholera by 2030, because the Crystal® VC RDT is used for rapid detection of *V. cholerae* in resource limited settings, and in health institutions (34). One of the limitations of the Crystal® VC RDT is that some results are demonstrated with faint test lines that might be observed as positive results which is one of the serious limitations of the test. In addition, the presence of lipopolysaccharide antigen in *V. cholerae* and other Gram-negative bacterial species contributes to false positive readings in the Crystal® VC RDT (17). However, it has been promising strength due to its sensitivity, rapid detection of the outbreak, user friendly and low cost in frequently cholera outbreak affected countries (35).

Conclusion and recommendations: The sensitivity and negative predictive value of the Crystal® VC RDT was high. Crystal® VC RDT is a user-friendly, rapid, equipment free option to use as a POCT. The use of Crystal® VC RDT for detection of *V. cholerae* in the facility and in field settings is therefore beneficial due to its comparative advantage over the culture method for being sensitive, low cost, and easy to use. In addition, this test uses for early detection of *V. cholerae* outbreak and epidemiological surveillance. Furthermore, the test uses to detect vaccine efficacy within one week. Therefore, our study suggests that to use Crystal® VC RDT for the preliminary detection of cholera during the occurrence of the outbreak. Furthermore, health professionals are recommended to use Crystal® VC RDT to detect cholera in order to provide immediate patient management.

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Author's contribution: AB, AG, YW and BG conception, design and analysis of the study; A.B collected the data and performed laboratory investigation, statistical analysis, interpretation of the results and manuscript writing. BG, AG and YW involved in manuscript draft writing, statistical analysis, approved quality of the data and interpretation of the results. BY, MT, AA, GY, GT, MB, TB, MA, and AM contributed in the manuscript writing, statistical analysis and interpretation of the results. All authors participated during manuscript writing. BG and AG edited the final manuscript and approval the version to be published. All authors read and approved the final manuscript.

Abbreviations: ACAPS: Assessment Capacities Project, Crystal VC: Crystal *Vibrio Cholerae*, LPS: Lipopolysaccharide, RDTs: Rapid Diagnostic Methods, TCBS: Thiosulphate citrate bile salt sucrose, WHO: World Health Organization

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Assessment of the Impact of Energy Drink Consumption on Nutritional Status of Undergraduate Students in Ogun State, Nigeria

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Abstract

Background: *Energy drink consumption is increasingly common among adolescents and young adults, particularly university students in Nigeria. The potential nutritional implications of these beverages, which are often high in sugar and calories, warrant investigation.*

Objectives: *This study aimed to assess the impact of energy drink consumption on the nutritional status of undergraduate students in Ogun State, Nigeria, with particular focus on its contribution to daily caloric intake and potential associations with Body Mass Index (BMI).*

Method: *A descriptive cross-sectional study was conducted between April and June 2024 among undergraduate students at a federal tertiary institution in Ogun State. A multi-stage sampling technique was used to select 250 students across 10 academic departments. Data were collected using a validated, semi-structured, self-administered questionnaire addressing socio-demographic characteristics, energy drink consumption patterns, and influencing factors. Anthropometric measurements were obtained following standardized procedures, and BMI was calculated. Data were analyzed using SPSS version 26.0, with statistical significance set at $p < 0.05$.*

Result: *The majority of respondents (44.4%) were aged 20-22 years, followed by 23-25 years (26.8%), 17-19 years (21.2%), and ≥25 years (7.6%). BMI categorization showed that 74%were within the normal weight range, 11.2% were underweight, 12% were overweight, and 2.8% were obese. Regarding energy drink consumption, 32.8% reported intake several times a month, 14.8% consumed once per week, and 7.2% consumed daily. The variety of available flavors (22%) were a key motivator for consumption. No statistically significant association was found between frequency of energy drink consumption and BMI categories ($p = 0.051$).*

Conclusion: *Energy drink consumption is prevalent among university students in Ogun State, driven in part by flavor preferences. However, this study found no significant short-term impact on nutritional status as measured by BMI. Longitudinal studies are recommended to assess potential long-term health effects of regular energy drink consumption.*

Introduction

Over the past three decades, there has been a global increase in the rate of overweight and obesity. While genetic factors may contribute to the development of obesity, the recent dramatic increase in the rate of obesity suggests that behavioral and environmental factors have also played a role (1). The most frequent causes of weight increase are dietary and lifestyle modifications, but new studies suggest that beverage additives may also be a significant factor (2). This nutritional shift affects undergraduate students, the majority of whom are late adolescents and young adults (3). According to Omage and Omuemu (4), the shift from adolescence to adulthood is a crucial time for the establishment of behavioral patterns that influence chronic diseases and long-term health. The majority of Nigerian undergraduate students do not have optimal nutritional condition; in fact, immediate attention is required. Since they make up the majority of Nigerians, undergraduates—adolescents and young adults—are probably going to be the most impacted (5).

Energy drinks in this study refer to non-alcoholic or mildly alcoholic beverages that contain stimulants such as caffeine, taurine, guarana, ginseng, B vitamins, and sugar or artificial sweeteners. They are marketed to boost energy, enhance alertness, and improve physical and mental performance. Energy drinks, both domestically and imported, are popular among adolescents as well as young adults in Nigeria, particularly university students (6). Energy drinks are widely available and suitable for all age groups, as their sale is not currently subject to tight regulations. However, in some countries, their sale has been prohibited due to concerns regarding their high caffeine content (7). According to Picard-Masson *et al.*, (8) these rules may include labeling products with a "high caffeine content," recommending a daily maximum intake, stating that energy drinks shouldn't be combined with alcohol, or even outright forbidding their sale. There is not much information available on the consumption patterns of energy drinks in Nigeria or how they could influence customers' caloric intake. Therefore, this study ascertains the possible contribution of frequently used energy drinks on the amount of calories required by undergraduate students in Ogun State's higher education institutions.

Method

Study area, design and period

The consumption of energy drinks and nutritional status among undergraduate students attending federal government-owned universities in Ogun State were examined using a cross-sectional and descriptive study methodology. This study was carried out in Ogun State, Nigeria. Ogun state lies in the south west part of Nigeria. It has 20 local governments with a total of 3,751,140 (2006 census). The study engages with students from 10 colleges with different departments and programs under them. The target population for this study consisted of only undergraduates in the institution and were assessed during their second semester.

Inclusion and exclusion criteria

Inclusion Criteria: Undergraduate students enrolled in full-time academic programs at the selected federal tertiary institution in Ogun State. Students who were available and willing to participate during the second semester of the 2023/2024 academic session. Also, respondents who provided informed written consent after being fully briefed on the objectives and procedures of the study.

Exclusion Criteria: Students who declined to participate or withdrew their consent at any stage of the data collection process. Incomplete questionnaires or improperly recorded anthropometric data were excluded from final analysis to ensure data quality and reliability.

Sample Size determination and sampling technique

The sample size (N) for this study was determined using the formula: $N = Z^2 \times P \times q \div d^2$, where the confidence interval (Z) is 1.96 (a constant), and the degree of freedom (d), representing the margin of error, is 0.05. The prevalence (P) of underweight among undergraduates in Ogun State is 13.4% (20), which when converted gives $P = 13.4 \div 100 = 0.134$. Consequently, $q = 1 - P = 1 - 0.134 = 0.866$. Substituting these values into the formula: $N = (1.96)^2 \times 0.134 \times 0.866 \div (0.05)^2$, we get $N = (3.841 \times 0.134 \times 0.866) \div 0.0025 = 0.44573 \div 0.0025 = 178.29$. To account for possible attrition, a total of 250 respondents were included in this study.

A multi-stage sampling technique was used to choose 250 undergraduate candidates in total. Out of the ten (10) colleges, five (5) colleges were chosen at random for the first stage.

In the second round, two departments were chosen at random from each of the five colleges. Two (2) departments were chosen from each of the five (5) colleges, and twenty-five (25) students were chosen at random from each of the ten (10) selected departments.

Data collection

Data collection was conducted by a team of four, two (2) trained research assistants who were final-year students in the department of Nutrition and Dietetics and the principal investigators who are the authors. Prior to fieldwork, the assistants underwent a one-day orientation and training session facilitated by the principal investigators. This training focused on the purpose of the study, ethical considerations, proper administration of the questionnaire, anthropometric measurement techniques, and respondent engagement strategies to ensure uniformity in data gathering. The questionnaire was pre-tested on a sample of 20 undergraduate students from a neighboring tertiary institution (college of education) but not included in the main study. Based on feedback, minor revisions were made to improve the structure and comprehensibility of certain items. The language of administration was English, as it is the official language of instruction in Nigerian tertiary institutions. Data collection took place during the second semester, which was strategically chosen to ensure that students were settled on campus and available to participate. Respondents completed a validated semi-structured, self-administered questionnaire that captured sociodemographic characteristics, energy drink consumption patterns, and other lifestyle-related variables. We measured the anthropometric assessment, participants' weight and height were measured using a calibrated digital weighing scale and a stadiometer, respectively. All measurements were taken twice and the average value recorded to minimize measurement errors. Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). All instruments were regularly checked for accuracy and zero error during each day of data collection. An energy drink consumption pattern and frequency questionnaire, adapted from Hasan et al., (9) was used to gather data from the respondents.

Data analysis

Before analysis, the results from the questionnaire were coded, and sorted, and then descriptive statistics employing frequencies and percentages were used to examine the data. While mean standard deviation was used to communicate continuous variables, frequency and percentages were used to express categorical data. The null hypothesis was tested using inferential statistics (Chi square) at the $p<0.05$ level of significance. The data analysis was conducted using SPSS 26.0, the Statistical Package for Social Sciences.

Ethical consideration

The questionnaires were administered to respondents on approval by ethical committee of the Ogun state hospital, Ijaye Abeokuta, reference Number: SHA/RES/VOL.23/ 079. Before collecting the data, informed consent of respondents was obtained and purpose of the study was explained to them. Participation in the study were voluntary and a respondent had the right to withdraw if he/she wishes to do so. Information provided was treated confidentially and respondent's anonymity was adequately maintained.

Result

Socio-Economic and Demographic Characteristics of the Respondents

Several key socio-demographic factors were looked at, providing insight into the respondents' varied traits. There was a noticeable variance in the respondents' age distribution. A significant percentage, 44.4%, was between the ages of 20 and 22, while 26.8% was between the ages of 23 and 25. A smaller but significant group consisted of people who were 17–19 years old (21.2%), and people who were 25 years old and older (7.6%). The respondents' varied age distribution reflects a wide range of life phases. There was an almost equal distribution of genders, with 51.6% of men and 48.4% of women. The dataset is guaranteed to provide a comprehensive perspective by the balanced gender representation. The interviewees' religious affiliations were primarily Christian (69.6%), with Islam coming in second at 30%. Just 0.4% of people identified as members of a faith other than their own. This distribution demonstrates how diverse the population under study was in terms of religion. The respondents' intellectual backgrounds ranged widely throughout various educational stages. The larger (38.8%) population were in their fourth year, followed by the first year students (24.4%), the second year students were 17.6 percent, the fifth year students were 11 percent, while the third year students were7.6 percent of the population. There is a range of seniority levels among the participants, as seen by this uneven distribution. In

terms of accommodation, 31.2% of respondents lived on campus, while 68.4% of respondents lived off campus. Just 0.4% of respondents said they lived with their parents or other family members. The distribution of houses highlights the respondents' varied living arrangements. When it came to extracurricular involvement (involvement in sports, clubs and other activities outside the class room), 26.8% of respondents said they participated in extracurricular activities, while 73.2% said they did not. This discrepancy in respondents' extracurricular involvement reflects their varied interests and activities outside of the classroom and indicates different levels of engagement.

Anthropometric Characteristics of the Respondents

The anthropometric characteristics of the respondents, providing valuable insights into their physical measurements and overall body composition. The average weight of the respondents was 61.304 kg, indicating the central tendency of the weight distribution. BMI, calculated from weight and height in table 1, provides an assessment of body fatness.

Table 1: BMI Category of the Respondents

BMI Category	Frequency	Percent
Underweight (BMI < 18.5 kg/m^2)	28	11.2
Normal (BMI: 18.5 – 24.9 kg/m^2)	185	74
Overweight (BMI: 25.0 – 29.9 kg/m^2)	30	12
Obese (BMI > 30 kg/m^2)	7	2.8
Total	250	100

Consumption Pattern and Frequency of Consumption of Energy Drinks among the Respondents

This sheds light on the respondents' varied patterns of energy drink usage and provides a thorough picture of their routines, frequency, amounts, and related behaviors. Thirty-one percent of the respondents did not drink energy drinks at all, indicating a sizeable section of the population that does not drink this product. The consumers' frequency of consumption varied: 32.8% indulged several times a month, 14.8% chose to consume once a week, while 7.2% of the respondents, a smaller but significant group, indicated daily consumption, indicating that occasional rather than habitual ingestion was more common in the population questioned.

When it came to quantity, the statistics showed that 39.6% of people only drank one can of energy drink a day, while 9.2% drank two. The majority of respondents moderated their weekly consumption, as seen by 54.4% of them who lowered their intake to fewer than five drinks per week. When the timing of consumption was examined, the results showed a variety of preferences. Energy drinks were commonly ingested in the afternoon (36.4%) and evening (24%), which is consistent with their possible use as study aids or supplies of evening energy. The percentage of people who drank energy drinks in the early morning (3.2%) and midday (5.2%) was lower than the overall percentage, suggesting that this was not a frequent practice.

Convenience stores were preferred by 59.2% of respondents based on their purchasing habits, demonstrating the accessibility and availability of energy drinks in these establishments. A concentration of sales in physical convenience stores was indicated by the lesser share of purchasing channels accounted for by supermarkets, internet merchants, and other sources. About 15.2% of respondents coupled energy drinks with alcohol or other substances, which is a worrying finding because it suggests that there may be health hazards involved with combination intake. A range of use durations was also noted, with 14.8% of respondents having drank energy drinks for 4-6 years and 22.4% of respondents for 1-3 years, indicating a consistent pattern of consumption among these groups. Finally, the amount spent on energy drinks varied; 42.4% of users spent less than $1 a week, suggesting that a sizable percentage of the market can afford it. On the other hand, 18.4% of the segment's weekly spending was in the$1–$2, indicating a significant amount of money spent on this beverage. The frequency of energy drink consumption among the respondents in table 2 was recorded, indicating the respondents' preference for some brands over others.

Table 2: Frequency of Consumption of Energy Drinks Among the Respondents

Energy Drink	Never	Everyday	1-3 times/week	4-6 times/week	More Than Once Per Day
LUCOZADE BOOST	49%	14%	16%	14%	6%
LUCOZADE ORIGINAL	20%	20%	26%	18%	16%
EVIRON (Can)	32%	18%	19%	17%	14%
CLIMAX (Can)	16%	26%	20%	26%	12%
CLIMAX (Bottle)	27%	8%	42%	10%	12%
CHI VERA(Can)	36%	12%	16%	26%	10%
POWER HORSE (Small)	19%	27%	17%	26%	11%
LUCOZADE SPORT	32%	9%	26%	24%	8%
FEARLESS	37%	14%	22%	17%	10%
POWER HORSE (Big)	40%	12%	20%	17%	11%
MONSTER	20%	15%	40%	12%	12%
MACA	12%	15%	22%	40%	10%
SUPA KOMANDO	18%	40%	14%	20%	8%
PREDATOR	20%	12%	14%	14%	40%
BULLET	10%	20%	45%	14%	10%
RED BULL	19%	19%	13%	43%	6%

Factors Influencing Consumption of Energy Drinks

This provides a more detailed knowledge of the intricate decision-making processes and reasons why the respondents consume energy drinks. One of the main motivators is the diversity of flavors available (22%) which suggests that customers have a preference for a wide range of taste experiences. This desire for a variety of flavors implies that the energy drinks' sensory appeal has a big impact on their decision. Simultaneously, health and safety concerns (10.8%) are reasons, representing a segment of the consumer base that places a high value on their health and takes into account any possible risks related to these drinks. Analyzing the main driving forces behind consumption, the data shows a complex picture. Enhancing energy and alertness (12.4%) shows that there is a need for these drinks in everyday life and that people seek them out for their practical advantages. Furthermore, a hedonistic component can be seen in the desire for taste and enjoyment (12.4%), which highlights the importance of pleasure and sensory experience in influencing consumption decisions.

Moreover, the examination of nutritional information (21.6%) shows a discriminating consumer base that actively interacts with the drinks' composition and content. This implies a degree of health consciousness, where people base their decisions on the nutritional content of the drinks. Furthermore, the fact that 45.6% of respondents were aware of the possible health hazards connected to energy drinks suggests that a sizable portion of the population was knowledgeable about these issues. This knowledge, along with the fact that a sizable percentage (44.8%) is worried about these hazards, emphasizes the need for more focused education and awareness efforts about the possible consequences connected to consuming excessive amounts of energy drinks.

The impact of marketing and advertising (24%) highlights the ability of marketing methods to persuade changes in consumption habits. This research highlights the necessity of responsible marketing strategies and laws to prevent consumers from being overly persuaded to make decisions that could endanger their health.

Relationship between Consumption Pattern of Energy Drinks and Anthropometric Characteristics

This presents the relationship between the frequency of consumption of energy drinks and different Body Mass Index (BMI) categories, including underweight, normal weight, overweight, and obese. The p-value associated with this rela-

tionship is 0.051, indicating that there is no statistically significant relationship between the frequency of energy drink consumption and BMI categories ($p > 0.05$). It was observed in table 3, that individuals with varying BMI categories consume energy drinks at different frequencies. For instance, individuals with normal weight tend to consume energy drinks more frequently across all categories compared to underweight, overweight, or obese individuals. However, the differences in consumption frequency among different BMI categories are not statistically significant according to its p-value.

Table 3: Relationship between Consumption Pattern of Energy Drinks and Anthropometric Characteristics

	BMI Category				p-value	Decision
Frequency of Consumption	**Underweight**	**Normal**	**Overweight**	**Obese**	0.051	NS
Daily	0%	6%	1%	0%		
Once per week	0%	14%	1%	0%		
Several times a month	4%	21%	5%	2%		
Rarely	3%	9%	2%	0%		
Never	3%	24%	4%	1%		

NS: Non-significant

Discussion

Energy drinks are non-alcoholic beverages with the promise of providing an extra energy boost for daily tasks. These are carbonated drinks with high levels of sugar and caffeine combined with mixes of unusual botanical extracts, B vitamins, and amino acids to provide short-term energy and mental clarity boosts for users. This study's objective was to evaluate undergraduate students at a government-owned university in Ogun State's energy drink intake and its impact on their nutritional status. The majority of respondents in this study are of normal weights while less than one-quarter are underweight. This is in agreement with results from other studies (10, 11).

The findings of this study, along with the research conducted by Mohammed (10) and Christina (11), do not come as a surprise considering that obesity has been identified as a growing issue among adult populations in developing nations. The rising incidence of obesity-related chronic disorders makes this trend unacceptable. When used frequently in addition to regular meals, energy drinks represent a major source of additional energy that may cause unintended weight gain (6, 12).

According to this study, which included over half of the respondents, energy drink intake is prevalent among undergraduates. Energy drinks are now readily available and accessible in nearby stores as well as on college campuses thanks to the quick growth in production and marketing. An increasing number of teenagers and young people are consuming energy drinks these days. Research by Sanctis *et al*. (13) and Yunusa *et al*. (14) revealed a similar pattern, with each study reporting a significant prevalence of energy drink (41.1% and 66.1 % respectively) intake among its participants. Additionally, a study done among the University of Port Harcourt medical and dental students revealed an alarmingly high prevalence of energy drink (80.1%) intake (15) while Nuss *et al.* (16) documented the opposite event at 24% energy drink consumption

When evaluating the frequency of energy drink intake, the study's weekly energy drink consumption is comparable to that of Christina *et al*. (11) who found that 23% of respondents drank energy drinks weekly. However, when compared to daily intake, the study participants' weekly consumption is higher. When compared to Ernesto Cabezas-Bou *et al.* (17) this report is also higher.

A broader comprehension of the complicated decision-making procedures and incentives underlying energy drink intake among the participants in this investigation demonstrated that the flavor experienced from consuming energy drinks is a determinant in their selection of energy drink consumption, with price being of little consequence as long as

the taste fulfilled their expectations. Similar results on respondents' preference for energy drinks influenced by taste were noted in (10). But according to Mohammed *et al.* (10) the cost was just as significant as flavor when selecting energy drink brands.

This study documented the respondents' practices of mixing energy drinks and alcohol; however, Ibrahim *et al*. (18) study found that fewer respondents in Kano acknowledged doing the same. This might be the case because respondents are unlikely to acknowledge drinking alcohol even if they do so to comply with local cultural standards. After all, it is considered a sacrilege and not an acceptable beverage in Kano.

Because they tend to improve alertness, job, and academic performance as well as health and social interactions, energy drinks are seen as advantageous (7). The benefits that participants in this study expected to experience from consuming energy drinks were staying awake, increasing energy and alertness, and enhancing physical performance. A sizable portion of respondents cited increased energy and alertness as their main motivation, which suggests that there is a need for these drinks in everyday life and that customers seek them out for their practical advantages. Another significant factor influencing the respondents' decisions in this study was peer pressure, as 34% of them admitted to being persuaded to drink energy drinks by their peers. According to a previous study (19), college students frequently drank energy drinks, especially if they didn't get enough sleep, needed extra energy generally, needed it for big course projects or exam preparation, or needed it while driving for extended periods of time. This result was also observed in this study, when a sizable percentage of the participants used energy drinks to increase their energy and alertness as well as to stay awake for their academic endeavors.

Conclusion

One of the study's limitations was that the respondents' nutrient intake was not evaluated to determine whether or not they were replacing their meals with energy drinks. This could have affected the respondents' anthropometric indices, particularly their weight gain, given that the majority of the respondents had normal body mass indices. Refreshment and flavor were found to be motivators for respondents' high-energy drink use in this study. Since a sizable portion of survey participants admitted to combining energy drinks with other drugs and alcohol, focus groups may be used in future research to better investigate the potential usage of energy drinks with other drugs and alcohol. Despite the high prevalence of energy drink consumption among students, the study found no statistically significant relationship between consumption frequency and BMI categories ($p = 0.051$). This suggests that while energy drink consumption is prevalent, it may not directly impact weight status in the short term. Given the potential long-term health implications of frequent consumption, further research is recommended to explore its broader effect on dietary habits and health.

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Antimicrobial profile of blood culture isolates of Enteric fever pathogens at tertiary care teaching hospital of Western Uttar Pradesh, India

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Abstract

Background: *Enteric fever is remains a significant public health concern globally, with its impact exacerbated by the rise of antimicrobial resistance, which is largely driven by indiscriminate and irrational antibiotic use. Continuous surveillance of local antimicrobial resistance trends is essential to guide effective treatment protocols and curb the spread of drug- resistance strains.*

Objective: *This study aimed to determine the prevalence, antibiotic sensitivity patterns, and extended-spectrum beta-lactamase (ESBL) production in culture-confirmed enteric fever cases caused by Salmonella enterica.*

Method: *A retrospective, laboratory record-based cross-sectional study was conducted at a rural tertiary care teaching hospital. Blood culture data from July 2017 to June 2019 were reviewed to identify S. enterica isolates and assess their antibiotic susceptibility patterns. Data were analyzed using Microsoft Excel 2010 and summarized using descriptive statistics (frequencies and percentages). The chi-square test was applied to determine statistical significance, with p-value < 0.05 considered significant.*

Result: *Out of 512 blood samples processed for culture and sensitivity, 35 (6.8%) yielded Salmonella species Isolates. Salmonella typhi accounted for (n = 30, 86%), followed by Salmonella paratyphi A (n = 5, 14%). S. typhi isolates showed 100% susceptibility to Imipenem, > 90% susceptibility to third-generation cephalosporins, and high susceptibility to Aztreonam (90%), Cefepime (90%), Levofloxacin (86.67%) and Ciprofloxacin (70%). S. paratyphi A strains showed complete susceptibility (100%) to Cefixime, Ceftazidime, Ceftriaxone, Amikacin, and imipenem, and 80% susceptibility to Levofloxacin, Cefotaxime, Cefepime, and Azatreonam. A low level of multidrug resistance was observed, but resistance to Nalidixic acid was notably high.*

Conclusion: *The findings highlight the importance of performing blood cultures and antibiotic susceptibility testing in all suspected enteric fever cases. The emergence of antimicrobial resistance underscores the urgent need for antimicrobial stewardship programs to regulate and rationalize antibiotic use and prevent the spread of multidrug-resistance Salmonella strains.*

Introduction

Enteric fever is a globally prevalent communicable disease involving multiple systems, caused by *Salmonella enterica*, subspecies enterica serovar typhi, and serovars paratyphi A, B & C [1].It is a serious issue of public health concern with an annual incidence rate exceeding 20 million cases and 2 lakh deaths [1].The situation has further worsened with the emergence of multidrug resistance in the enteric fever pathogens which are now reportedly resistant even to first-line antibiotics like Chloramphenicol, Ampicillin & Co-trimoxazole [2].This has resulted in irrational injudicious drug therapy leading to selection pressures attributed to a single large self-transferable plasmid [3].This in turn has resulted in an increase in the usage of Fluoroquinolones like Ciprofloxacin in clinical settings. This has resulted in a gradual rise in Minimum inhibitory concentrations of Ciprofloxacin causing therapeutic failure [3]. This problem is worsened due to the emergence of Nalidixic acid-resistant *S. typhi* (NARST) strains which are also found to be resistant to Fluoroquinolones [4, 5].

As per the existing trends, 3rd generation Cephalosporins (Ceftriaxone, Cefixime), and macrolides (Azithromycin) are the preferred therapeutic agents for Enteric fever. However, with their increasing use, resistance against these antibiotics is increasingly reported among *S. enterica* strains [6].The emerging Multidrug resistance (MDR) in these strains has resulted in treatment failures, complications, increased risk of fecal-oral transmission, and a significant rise in morbidity and mortality [7]. Regular periodic monitoring of local antimicrobial resistance trends is a prerequisite for implementing rational measures and updating the therapeutic guidelines [8].

Given the above facts, we undertook this study to assess the prevalence antimicrobial susceptibility pattern and ESBL production pattern of Enteric fever pathogens derived as blood culture isolates at a rural tertiary care centerin western Uttar Pradesh.

Method

This laboratory data-based retrospective cross-sectional was conducted in the Department of Microbiology of a rural tertiary care center of western Uttar Pradesh, India wherein the Laboratory data of blood culture-positive cases of Enteric fever maintained over 2 years from July 2017 to June 2019 was retrieved, reviewed, and analysed to determine the prevalence of culture-proven Enteric fever cases and the antibiotic susceptibility pattern of *S. enterica* isolates. This study was undertaken after seeking approval for the conduct of the study from the Institutional Ethics Committee.

Study population: Our study population comprises of patients attending the Out patients department (OPD) of the hospital with complain of fever.

Inclusion and exclusion criteria

Inclusion criteria: Samples for blood culture collected from clinically suspected cases of Enteric fever before administering antibiotics

Exclusion criteria: Patients already on antibiotics.

Sample size calculation and sampling technique

Sample size was calculated using the formula Sample size = $4pq/d^2$ where p is the prevalence, q is 100-p and d is the precision (acceptable level of error which is 0.05 at 95% confidence interval).

Sampling technique: Purposive sampling

Collection of Blood samples

10 ml venous blood from adult patients and 5 ml from pediatric patients (clinically suspected cases of Enteric fever) were collected aseptically before starting any antimicrobial and inoculated into respective blood culture bottles containing BHI Broth with SPS (Microexpress, India) respectively and transported immediately to Microbiology Laboratory [9].

The inoculated blood culture bottles were incubated aerobically at 37^0C for 24 hrs. Subcultures were made on blood agar and MacConkey agar plates every alternate day till the 7th day [9]. The pale-coloured colonies grown on MacConkey agar depicting Non-lactose fermenters (NLF) were further processed. Isolation, Identification, and characterization of *Salmonella sp.* were done using standard microbiological techniques and biochemical tests followed by Slide agglutination using antisera [10, 11]. A set of following antibiotic discs was applied on a pre-seeded Muller-Hinton agar plate with 0.5 McFarland standard inoculums by modified Kirby Bauer's disc diffusion method and was interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. Ampicillin (10µg), Azithromycin (15µg), Ciprofloxacin (5µg),

Levofloxacin (5µg), Ceftriaxone (30 µg), Cefixime (5µg), Cefotaxime (30µg), Ceftazidime (30µg), Cefipime (30µg), Chloramphenicol (30µg), Cotrimoxazole (1.25/23.75µg), Nalidixic acid (30 µg), Tetracycline (30µg), Amikacin (30µg), Imipenem (30 µg), Aztreonam (30 µg) [Himedia Lab. Pvt Ltd, Mumbai, India]

Detection of extended-spectrum beta-lactamase

Isolates that were found to be resistant to at least two 3rd generation Cephalosporins like Cefotaxime (30µgm) Ceftazidime (30 µgm), Ceftriaxone (30 µgm), etc., were considered to be probable ESBL producers and further screened for ESBL production by Combined Disc diffusion Test as per CLSI 2016 guidelines using Antibiotic discs of Cefotaxime (30 µg) and Ceftazidime (30 µg), Cefotaxime clavulanate (30/10 µg) and Ceftazidime clavulanate (30/10 µg). More than5 mm increase in diameter of the inhibition zone of the Cefotaxime clavulanate and Ceftazidime clavulanate disc compared with the respective cefotaxime and ceftazidime disc alone was interpreted as phenotypic evidence of ESBL production [12].

Data analysis

Data collection and analysis were done using MS Office Excel 2010. Statistical analysis was done using descriptive statistics, presented as frequencies and percentages in tables and graphs. The chi square test was applied to determine the levels of significance (p-value <0.05 was considered significant).

Ethical considerations

The study was undertaken after seeking approval from institutional ethics committee, KD Medical College, Mathura (UP)-India (236/IECBMR/KDMC/2019). Confidentiality regarding the identity and personal information of the patients was maintained.

Result

A total of 512 blood samples were processed for culture sensitivity during 2 years out of which 35 clinical isolates of *Salmonella spp* (0.07%) were obtained. *Salmonella typhi* was predominant with 30 strains (86%) followed by *Salmonella paratyphi A* with 5 strains (14%).

The majority of cases of enteric fever were reported from the 21-30 years age group and least from 51-60 & >60 yrs. Age groups. In this study, Enteric fever cases exhibited male preponderance with male: female ratio being 2.2:1 for all *Salmonella* isolates with 2:1 for *S. typhi* and 4:1 for *S. paratyphi A* **(Table 1).**

Table 1: Age-wise distribution of culture-confirmed Enteric Fever cases

	Enteric fever N (%)		P- value
	Positive	**Negative**	
Sex			
Male	24	245	0.049
Female	11	232	
Age group (in yrs.)			
≤ 10	4	72	0.56
11-20	7	95	0.99
21-30	11	115	0.33
31-40	5	62	0.83
41-50	4	49	0.83
51-60	2	48	0.40
> 60	2	38	0.60

Significance calculated using chi square test with level of significance set at p< 0.05

S. typhi isolates exhibited high sensitivity towards Imipenem (100%) followed by third-generation cephalosporins [Ceftriaxone (96.67%), Ceftazidime (96.67%), Cefixime (93.33%), Cefotaxime (90%)], Monobactums like Aztreonam (90%) and fourth generation Cephalosporins like Cefepime (86.67%). The *S. typhi* isolates were least susceptible to Nalidixic acid with 76.67% of test strains being resistant followed by Tetracycline with a 50% resistance rate **(Fig 1, Table 2).**

The susceptibility pattern of *S. paratyphi A* isolates as depicted in Table 2 and Fig.2 showed the highest susceptibility towards Cefixime, Ceftazidime, Ceftriaxone, Amikacin & Imipenem(100%) followed by Levofloxacin, Cefotaxime, Cefepime and Aztreonam (80%). The highest resistance rates were seen against Nalidixic acid and Azithromycin. Amongst fluoroquinolones, Levofloxacin exhibited modest sensitivity against *S. typhi* and *S. paratyphi* (86.67% and 80%, respectively) (**Fig 2, Table 2).**

Table 2: Antibiotic susceptibility profile of Enteric fever pathogens

Antibiotics tested	*S. typhi* (30)	*S. paratyphi A* (5)	*P-value*
Ampicillin	22 (73.33)	3(60)	0.54
Azithromycin	23 (76.67)	2 (40)	0.09
Ciprofloxacin	21 (70)	3 (60)	0.65
Levofloxacin	26 (86.67)	4 (80)	0.69
Ceftriaxone	29 (96.67)	5 (100)	-
Cefixime	28 (93.33)	5 (100)	-
Cefotaxime	27 (90)	4 (80)	0.5
Ceftazidime	29 (96.67)	5 (100)	-
Cefepime	27 (90)	4 (80)	0.5
Chloramphenicol	24 (80)	4 (80)	1.0
Cotrimoxazole	21 (70)	4 (80)	0.65
Nalidixic acid	7 (23.33)	1 (20)	0.87
Tetracycline	15 (50)	3 (60)	0.68
Amikacin	26 (86.67)	5 (100)	-
Imipenem	30 (100)	5 (100)	-
Aztreonam	28 (93.33)	4 (80)	0.32

Significance calculated using chi square test with level of significance set at p< 0.05

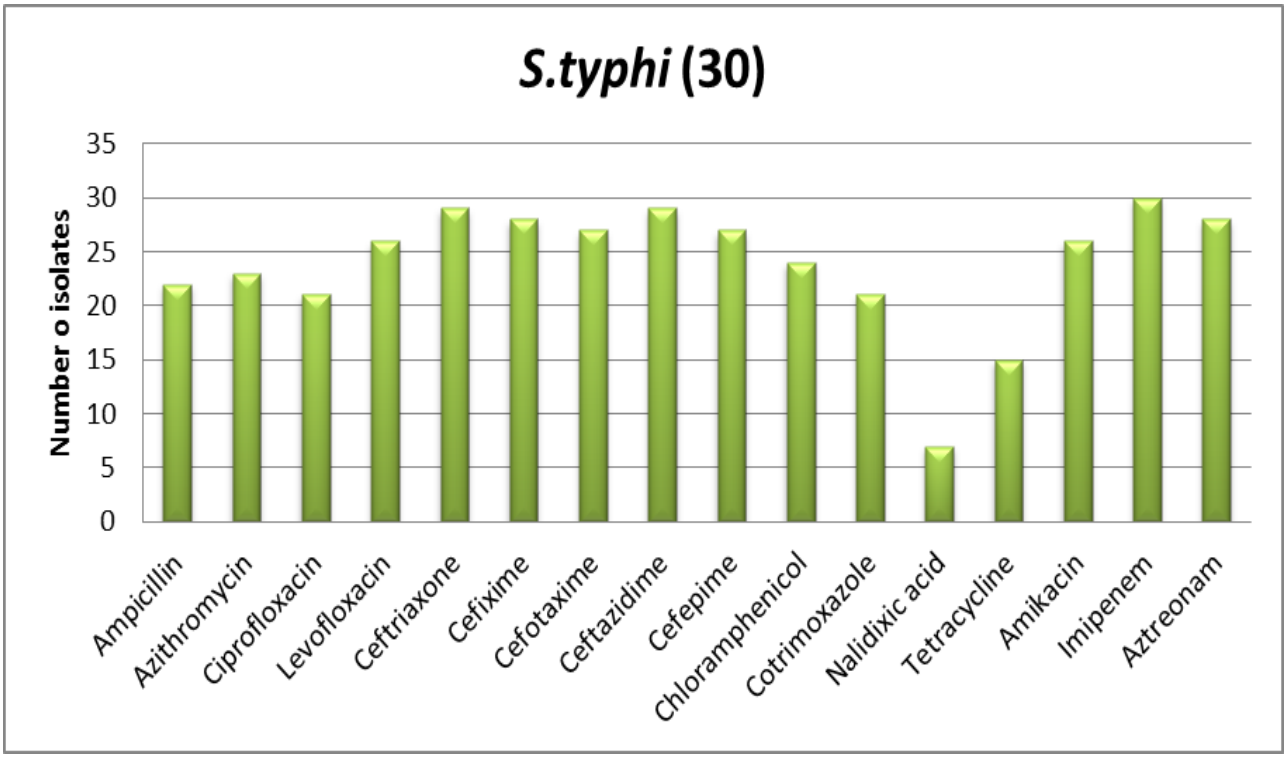

Figure.1: *Antibiotic Susceptibility Pattern of S. typhi strains*

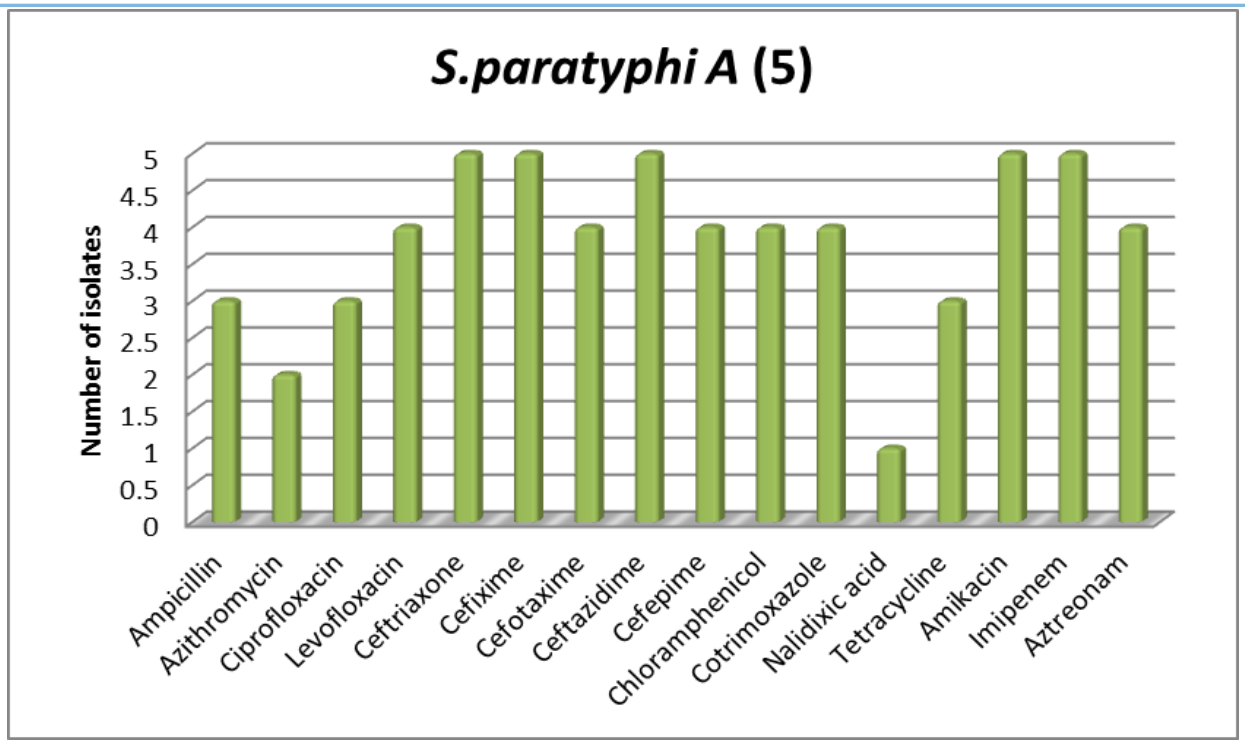

Figure 2: *Antibiotic Susceptibility pattern of S. paratyphi Astrains*

Amongst the 35 isolates, multidrug resistance towards first-line drugs was seen in 6 isolates (17 %) all being *S. typhi* strains. All these MDR strains were also resistant to Nalidixic acid (MDR-NAR). The majority of the test isolates were found to be resistant to Nalidixic acid (NARST) but predominantly susceptible to fluoroquinolones like Levofloxacin. In this study, a much higher Azithromycin resistance rate was seen for *S. paratyphi A* as compared to S. typhi with 60% of strains showing resistance. There was no significant difference in the antibiotic sensitivity pattern of *S. typhi* and *S. paratyphi* A strains (P = 0.325). Some of the important limitations of this study are that the antibiotic susceptibility pattern of *Salmonella spp.* was derived by Standard disc diffusion test with results not confirmed by MIC determination; and that it is a single-centre retrospective study with a limited sample size.

None of the isolates came out to be positive for ESBL production as screened by the combined disc diffusion test (CDST).

Discussion

Amongst the Enteric fever pathogens isolated in our study, *Salmonella typhi* was predominant followed by *Salmonella paratyphi A*, like several similar previous studies [4, 13- 16]. The majority of cases of enteric fever were reported from youngsters in the 21-30 years age group and least from the elderly as reported in a number of similar studies [8, 10, 17-19]. Like our study, several similar studies in the past have also reported male preponderance in culture-confirmed cases of enteric fever [8, 10, 14, 16, 18, 20, 21]. This is probably due to the high vulnerability of males owing to more outdoor exposure.

In our study, *S. typhi*i solates exhibited the highest sensitivity towards Imipenem followed by third-generation cephalosporins, and the least sensitivity to Nalidixic acid and Tetracycline. Similar findings were reported by many studies [16, 19, 22- 28]. Amongst fluoroquinolones Levofloxacin exhibited modest sensitivity against *S. typhi* and *S. paratyphi* (86.67% and 80% resp.) but when compared to previous studies resistance against fluoroquinolones esp. Ciprofloxacin is following an increasing trend due to the selective pressure of unrestricted rampant usage as the mainstay of typhoid therapy [3, 29-33]. None of the isolates came out to be positive for ESBL production as screened by the combined disc diffusion test (CDST). Similar findings were reported previously [10].

Amongst the 35 isolates, multidrug resistance towards first-line drugs was seen in 6 isolates (17 %) all being *S. typhi* strains. All these MDR strains were also resistant to Nalidixic acid (MDR-NAR). These observations were in line with

many similar studies [10, 16-20, 34]. In this study multidrug resistance was not found amongst *S. paratyphi* strains which is consistent with the previous reports [3, 18, 19, 35, 36].

The majority of the test isolates were found to be resistant to Nalidixic acid (NARST) but predominantly susceptible to fluoroquinolones like Levofloxacin. However, it has been suggested that such strains (NARST) should be considered Fluoroquinolone resistant; Nalidixic acid being a surrogate marker to predict FQ failure as per CLSI guidelines. As Nalidixic acid resistance amongst *Salmonella* spp. is rapidly increasing in India, which may lead to the dilemma in the use of Fluoroquinolones considered to be one of the most effective drugs in Enteric fever treatment. But the consistent use of FQ esp. Ciprofloxacin in NA-resistant cases has led to a steady rise in MIC along with further mutations at the same locus which has led to the emergence of completely resistant strains.

In this situation, the Standard disc diffusion test could no longer be relied upon and only MIC determination by any of the available methods like an E-test should be preferred for detecting Ciprofloxacin resistance, particularly in all Nalidixic acid-resistant strains [10]. As per recent therapeutic guidelines for Nalidixic acid sensitive S. typhi (NAAST) strains, a 7-day regime and for NARST a 10-14 days high dose course is recommended [3, 30]. In the MDR-NAR cases, third-generation Cephalosporins and broad-spectrum azilide-azithromycin are potential treatment options. Azithromycin can achieve rapid remission, prevent relapse, and reduce fecal carriage rates through its high intracellular concentration and long elimination half-life. Indian Academy of Paediatrics task force on the management of enteric fever had recommended Azithromycin as an oral drug for uncomplicated enteric fever where initial first-line therapy has failed [3, 37, 38]. But in this study much higher Azithromycin resistance rate was seen for *S. paratyphi A* as compared to *S. typhi* with 60% of strains showing resistance [3, 35].

A low level of multidrug resistance but a high level of Nalidixic acid resistance was reported in this study like many other studies [3, 4, 6, 29, 38-41]. This study has shown re-emergence and an appreciable increase in susceptibility of *Salmonella enterica* strains towards first-line antibiotics attributed to a sharp decline in their usage by clinicians over the last decade resulting in the withdrawal of selection pressure [4, 6, 39]. Loss of self-transmissible plasmids and the emergence of de novo susceptible strains might be the other reasons for anticipating the possibility of reconsidering these drugs as potential therapeutic agents in Enteric fever [3,18,29].

This study has shown a very high susceptibility of test strains towards third-generation cephalosporins like other studies [6, 29]. So, these drugs are often considered as drugs of choice for enteric fever esp. in fluoroquinolone-resistant cases. However, the emergence of Extended-spectrum beta-lactamase and ACC-1 AmpC beta-lactamase-producing strains causing Enteric fever is a serious public health threat resulting from selection pressure due to injudicious inappropriate rampant usage of 3rd generation cephalosporins. With the high levels of resistance being reported against Fluoroquinolones and nalidixic acid this is an alarming situation as it could seriously limit therapeutic options. Therefore appropriate judicious selection and rotation of antibiotics guided by the knowledge of their susceptibility profiles is of utmost importance [3, 6, 16, 42].

Some of the important limitations of this study are that the antibiotic susceptibility pattern of Salmonella spp. was derived by Standard disc diffusion test with results not confirmed by MIC determination; and that it is a single-centre retrospective study with a limited sample size.

Conclusion

This study indicates that first-line antibiotics could now be re-incorporated into enteric fever therapy. It is recommended to determine MIC values for fluoroquinolones before therapy to avoid treatment failures. The third generation cephalosporins should be used judiciously with caution. This study emphasizes the need for blood cultures and antibiotic susceptibility testing for every suspected case of enteric fever. Injudicious, Irrational drug therapy must be restricted through antimicrobial stewardship measures. Appropriate Surveillance strategy for regular continuous monitoring of antimicrobial susceptibility patterns with the formulation of antibiotic policy at the institutional or regional level is an important prerequisite to rationalize enteric fever treatment protocols to curb the menace of rapidly emerging drug resistance amongst such pathogens. Apart from this, improving living conditions, creating public awareness regarding general hygiene, infection control practices, and appropriate

usage of typhoid vaccines will help in controlling typhoid in a community.

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Modeling Determinants of Time to Death of Stroke Patients in Harari Regional State, Ethiopia: An Application of Shared Frailty Models

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Abstract

Background: *Stroke, a condition caused by interrupted blood flow to the brain, is the second leading cause of death worldwide and a major contributor to morbidity and disability. Despite its global impact, there remains a need for more comprehensive and flexible statistical models to better understand the timing of stroke-related mortality. This study addressed this gap by applying robust shared frailty models to stroke patient data.*

Objective: *The main purpose of this study was to identify the factors influencing time to death among stroke patients using shared frailty models, accounting for hospital-level clustering effects.*

Methods: *A retrospective study was conducted at Harar Regional State, Ethiopia, across three hospitals: Harar General Hospital, Jegol hospital, and Hiwot Fana Specialized University Hospital. A total of 224 stroke patients admitted to medical wards between September 1, 2020, and November 1, 2023, were included. Data was coded, cleaned, and entered in to SPSS version 25 and further analyzed using R-Studio. The presence of clustering (frailty) effects among hospitals was evaluated, and different shared frailty models were compared to identify the best-fitting model.*

Results: *Of the total 224 stroke patients, 51(22.77%) died during the study period, while 173 (77.23%) were censored. The median survival time was estimated at 14 days, highlighting the acute nature of the disease. The Weibull-inverse Gaussian shared frailty model provided the best fit for the data, accounting for unobserved heterogeneity across hospitals. Significant predictors of shorter time to death included hypertension (φ = 2.118; 95% CI: 1.145-3.917), cardiac disease (φ = 2.667; 95% CI: 1.343-5.296), diabetes mellitus (φ = 3.035; 95% CI: 1.1.560-5.906), atrial fibrillation (φ = 3.247; 95% CI: 1.619-6.511), and presence of basic complications (φ = 2.983; 95% CI: 1.477-6.023).*

Conclusion: *This study highlights the importance of hospital-level clustering effects in survival analysis of stroke patients. The significant frailty effect suggests variability in outcomes across hospitals, underlining the need for tailored interventions. Clinicians and hospital administrators should consider these differences when managing stroke patients, emphasizing timely follow-up, individualized care, and resource allocation to improve survival outcomes.*

Introduction

A potentially deadly stroke may arise from an inadequate blood supply to the brain. Two types of strokes signify a halt to blood flow or bleeding: hemorrhagic and ischemic strokes. Dementia and depression can also result from strokes [1]. Stroke rates have already risen to epidemic levels. Worldwide, strokes have claimed the lives of nearly 110 million people. A stroke will occur in the lifetime of one in four adults over the age of 25. According to WHO data from 2022, around 12.2 million people worldwide will have a stroke for the first time, and the illness will be the cause of 6.5 million deaths [2].

Stroke is the world's second leading cause of death, after ischemic heart disease. The global burden of stroke, including mortality, morbidity, and disability, is rising [3]. In high-income countries, in-hospital mortality for stroke patients ranges from 3% to 11%, whereas in low-and middle-income countries it increase to 7%-15%. Hemorrhagic strokes carry a substantially higher fatality rate (nearly 38%) compared with ischemic strokes (8%-12%), with outcomes influenced by factors such as stroke severity, patient age, comorbidities, treatment efficacy, and complications [3]. Given the rising global burden of stroke, approximately one person worldwide suffers a stroke every two seconds [4]. According to the 2022 Global Stroke Fact Sheet, the lifetime risk of experiencing a stroke has increased by 50 percent over the past 17 years. Between 1990 and 2019, stroke incidence rose by 70 percent, mortality by 43 percent, prevalence by 102 percent, and disability-adjusted life years (DALYs) by 143 percent. Notably, low- and lower-middle-income countries shoulder the greatest portion of this burden, accounting for 86 percent of stroke-related deaths and 89 percent of stroke-attributable DALYs [5].

In Sub-Saharan African (SSA) countries, the range was 11.1% to 43.4%, three to four times higher than in developed nations [6]. While stroke rates have dropped by 42% in high-income nations, they have risen by over 100% in low- to middle-income countries. Research suggests that stroke incidence and prevalence show minimal geographic variation [7]. According to WHO data released in 2020, stroke caused 39,362 deaths in Ethiopia, accounting for approximately 6.98% of all deaths in the country. Ethiopia has an age-adjusted stroke mortality rate of 83.71 per 100,000 population, ranking 90thglobally. A study conducted at St. Paul's Millennium Medical College in Ethiopia reported an average hospital stay of nine days for stroke patients, with an in-hospital mortality rate of 14.7% (23.5% for hemorrhagic strokes and 6.1% for ischemic strokes) [8]. The 2013 Global Burden of Disease report indicates that strokes are responsible for 80% of mortality in low- and middle-income countries. Despite stroke consistently ranking among the top three causes of morbidity and mortality in Ethiopia in recent years, there is limited knowledge about the outcomes of stroke treatment when adequate resources are available [9].

In a frailty model, the acceleration factor (φ) quantifies the effect of a covariate on the time to an event. That means φ is equivalent value with a risk ratio of stroke patients and interpreted as 100 people per φ (risk ratio). It essentially represents how much faster or slower an individual experiences the event compared to a baseline, based on the value of the covariate. A value of φ greater than 1 indicates a faster time to event, while a value less than 1 indicates a slower time to event. These models are used in survival analysis, particularly when dealing with clustered or correlated data. They account for unobserved heterogeneity (frailty) that might influence the survival times of individuals within the same cluster [15]. This study aims to evaluate the heterogeneity of unobserved factors including a random effect (frailty) in the model. It focused on the risk factors of mortality among stroke patients treated at Jegol Hospital, Harar General Hospital, and Hiwot Fana Specialized University Hospital in the Harari regional state, Ethiopia. The main objective of this study was to model the determinants of the time to death among stroke patients using a shared frailty model.

Method

Study setting, design, and period

This study utilized a retrospective study design from September 1, 2020 to November 1, 2023 in the three hospitals of Harar City: Harar General Hospital, Jegol Hospital, and Hiwot Fana Specialized University Hospital. The city is located in eastern Ethiopia approximately 526 kilometers from Addis Ababa, the capital city of Ethiopia. Those hospitals are well-equipped to provide a quality healthcare services give with an infrastructure and ample supplies for the patients. Offering a comprehensive range of services from inpatient and outpatient care to emergency and specialized treatments

such as surgery, they play a crucial role in meeting the diverse healthcare needs of the population. Furthermore, the subsidies for healthcare services at these hospitals make them affordable and accessible to nearly all residents in Harar City.

Source and study population: A secondary source of a stroke patients' data were collected for this study. The population study was considered a patient who received a treatment from medical ward outpatient in the hospitals from September 1, 2020 to November 1, 2023.

Inclusion and exclusion criteria's

Inclusion criteria: Stroke patients who had been admitted to the intensive care unit ward at those hospitals were included in this study from September 1, 2020 to November 1, 2023. All patient charts with a complete data in the variables of interest were included in this study.

Exclusion criteria: In hospitals, stroke patients' charts were excluded from certain treatments or clinical trials due to specific criteria like the severity of the stroke, the patient's medical history, or time elapsed since the stroke's onset. Additionally, patients with certain medical conditions or those who don't meet the specific time windows for treatment was also be excluded.

Data collection methods and tools

A data collection was reviewed a stroke patients medical records, using tools structured data collection forms. The data collection tool was included patient demographics (sex, age and place of residence), disease types (hypertension, cardiac disease and diabetes mellitus), atrial fibrillation, baseline complication, stroke types, drug types, and patients' treatment from time-to-death. The following activities were done for a data collection methods and tools in the study. **Pre-testing the data collection tool:** Before the main study, the data collection tool was pre-tested on a small sample of records to identify any potential issues with clarity, applicability, or consistency of the variables. **Trained data collectors:** Trained medical professionals, such as nurses or residents, were involved in the data extraction process to ensure accurate and consistent data collection. **Retrospective review of medical records:** This involves carefully examined the existing patient charts and files were extracted a relevant information for stroke patients datasets. **Structured data collection forms:** These forms were designed to systematically gather specific data points from the medical records, ensuring consistency, and completeness.

Study variables

Dependent variable: Time to death measured (in days) from the start of anti-stroke treatment to the date of the patient's death or censoring. **Independent variables:** Sex, age, residence, hypertension, cardiac disease, diabetes mellitus, atrial fibrillation, baseline complication, stroke types, and drug types were considered under this study. The details of those variables, categories (codes), and data nature were described in the table below **(Table 1).**

Table 1: Descriptions of the variables, categories (codes) and data nature of stroke patients in Harari regional state from September 1, 2020 to November 1, 2023.

Variable	Levels and Codes	Data Nature
Sex	Female=0, Male=1	Categorical
Age	year	continuous
Place of residence	Urban=0, Rural=1	categorical
Hypertension	No=0, yes=1	categorical
Cardiac disease	No=0, yes=1	categorical
Diabetes mellitus	No=0, yes=1	categorical
Atrial fibrillation	No=0, yes=1	categorical
Baseline complication	No=0, yes=1	categorical
Stroke types	Ischemic=1, Hemorrhagic=2	categorical
Drug types	Anti-coagulants and Anti-platelet=1, Anti-coagulants, Anti-platelet and Anti-hypertensive=2, Anti-coagulants, Anti-platelet, and Statin=3, Anti-coagulants, Anti-platelet, Statin and Anti-hypertensive=4, Anti-coagulants, Anti-platelet, Statin, Anti-hypertensive and Antibiotics=5, Anti-platelet, Statin and Anti-hypertensive=6, Anti-hypertensive and Antibiotics=7, Anti-coagulants and Anti-hypertensive=8 and Other=9	categorical

Data management and analysis

A stroke patients' datasets was coded, cleaned, and entered in to SPSS version-25 for data management and R-studio version -4.3 for analyzed. The first step of the analysis was made a descriptive statistics that shown a frequencies and percentages of the variables. At second stage: a non-parametric test was used for compare groups of variables such as Kaplan Meier estimates, log-rank test and Wilcoxon test, whose P<0.05 were considered for multivariate analysis. In third step, fit a Cox-proportional hazard model to check a relationship between the dependent and independent variables. Finally, a marginal log-likelihood approach was used a parameter estimation to fit a robust model for shared frailty model was considered different frailty distributions and predicting.

The Survival models

The time until an event occurs is the outcome variable of interest in survival analysis, which is a collection of statistical processes for data analysis. The time variable in a survival analysis is commonly referred to as "survival time (ST)", since it indicates how long an individual has survived over a given period of time [10]. Let T be a random variable associated with the STs, t is the value of T, and $f(t)$ be the probability density function (PDF) of ST at a time value, t. A cumulative distribution function (CDF), $F(t), t \geq 0.$

$$F(t) = P(T \leq t) = \int_0^t f(u)du \qquad (1)$$

Survival and hazard function (SF & HF), and commutative hazard function (CHF) in the equation number 2, 3 and 4 were presented, respectively.

$$S(t) = P(T \geq t) = \int_t^{\infty} f(u)du \qquad (2)$$

$$h(t) = \frac{f(t)}{S(t)} = -\frac{d}{dt}\ln S(t) \qquad (3)$$

$$H(t) = \int_0^t h(u)du \qquad (4)$$

Non-Parametric Survival Model

Kaplan-Meier Estimator (KME): Suppose that r individuals have failures in a group of individuals and $0 \leq t(1) \leq t(2) \leq ... \leq t(r) < \infty$ be the observed ordered death times [11]. Assume that $r(j)$ be size of the risk at $t(j),$ where the risk set encompasses individuals alive and uncensored before $t(j).$ Let $d(j)$ be the number of observed events at $t(j), j = 1,2,...,r.$ Then, the KMEs for both survival and CHF had a probability of developing a disease at any time in equation 5 and 6 were presented, respectively.

$$\hat{S}(t) = \prod_{t(j)<t} \frac{[r(j) - d(j)]}{r(j)} \qquad (5)$$

$$H(t) = -\ln \hat{S}(t) \qquad (6)$$

Median Survival Time (MST): The smallest observed ST for which the value of the estimated SF is less than 0.5[12]. Where, $t(i)$ is an observed ST for the i^{th} individual, $i = 1,2,...,n.$

$$t(50) = \min \frac{t(i)}{S(t_i)} \leq 0.5 \qquad (7)$$

Frailty Model (FM): The random effects (frailty) element was added to standard models of analysis to account for unmeasured variables or linked survival data [13].

Shared Frailty Model (SFM): Conditional on the random term as a frailty denoted by $u_i,$ the STs in cluster-i $(1 \leq i \leq n)$ was assumed to be independent and the proportional hazard frailty model (PHFM) [14].

$$h_{ij}(t/x_{ij}, u_i) = h_0 \exp(\beta x_{ij} + u_i) \qquad (8)$$

Where, as an alternative, if the PHs assumption does not hold, then an AFTFM was applied.

$$h_{ij}(t/x_{ij}, u_i) = h_0 \exp(\beta x_{ij} + u_i)[\exp(\beta x_{ij} + u_i)t] \qquad (9)$$

Where, i is indicates the i^{th} cluster and j is indicates the j^{th} individual for the i^{th} cluster, $h_0(.)$ is the baseline hazard, u_i is the random term for all the subjects in cluster i, X_{ij} is a vector of the covariates for subject j in cluster i, and β is a vector of the regression coefficients.

Test of Unobserved Heterogeneity: The variance of θ is both large and statistically different from 0, indicates a cluster's heterogeneity, and the individuals' had a strong association to each other. Let θ=0, frailties=1, means that the cluster effects are not existent and the occurrences are independent for both within and between clusters [15].

Frailty Distribution

Gamma Frailty Distribution (GFD): The distribution of frailty Z is one of a parameter for GD [16]. The density of a GD of a random variable with parameter, $\theta > 0.$

$$f_Z(Z_i) = \frac{Z_i^{\frac{1}{\theta}} \exp(-Z_i/\theta)}{\Gamma(1/\theta)^{1/\theta}} \quad (10)$$

Where, $\Gamma(.)$ is GF and GD $(\mu, \theta),$ with μ is fixed to be one for identifiable and its variance becoming $\theta.$ Where, $Z_i > 1,$ individuals in the group-i are more frailty, $Z_i < 1,$ individuals are less frailty and have lower risk.

Conditional SF and HF of the GFD were given, respectively [17].

$$S_\theta(t) = [1 - \theta \ln S(t)]^{\frac{-1}{\theta}} \quad (11)$$

$$h_\theta(t) = h(t)[1 - \theta \ln S(t)]^{-1} \quad (12)$$

GD measures the association between any two event times from the same cluster in the multivariate case.

$$\tau = \frac{\theta}{\theta + 2}, \ where\ \tau \in (0,1) \quad (13)$$

Inverse Gaussian Frailty Distribution (IGFD): The PDF of an IND of a random variable with a mean of 1 and variance, $\sigma^2 = \theta$ [18].

$$f(Z) = \frac{1}{\sqrt{2\prod \theta Z^3}} \exp[\frac{-1}{2\theta Z}(Z-1)^2] \quad (14)$$

Where, $\theta > 0\ \&\ z > 0.$ Consequently, the Laplace transform (LT) of the IND.

$$L(s) = \exp[\frac{1}{\theta}(1 - \sqrt{1 + 2\theta s})];\ \theta\ and\ s > 0 \quad (15)$$

Conditional SF & HF were presented, respectively.

$$S_\theta(t) = \exp[\frac{1}{\theta}(1 - \sqrt{1 - 2\theta \ln S(t)})];\ \theta > 0 \quad (16)$$

$$h_\theta(t) = h(t)[1 - 2\theta \ln S(t)]^{-1/2};\ \theta > 0 \quad (17)$$

IGD frailty yields a Kendall's Tau.

$$\tau = \frac{1}{2} - \frac{1}{\theta} + 2\frac{\exp(2/\theta)}{\theta^2}\int_{\frac{2}{\theta}}^{\infty} \frac{\exp(-u)}{u} du;\ where, \tau \in (0, \frac{1}{2}) \quad (18)$$

Parameter Estimation

The ST of the random variables was given the covariate information from the marginal log-likelihood of the observed data [19].

$$Lm\arg(\varphi, \beta, \theta, Z, X) = \sum_{i=1}^{s}[\sum_{j=1}^{n_i} \delta_{ij}(\log(h_0(y_{ij}) + X_{ij}^T\beta)] + \log[(-1)^{d_i} L^{d_i}(\sum_{i=1}^{n_i} H_0(y_{ij})\exp(X_{ij}^T\beta))] \quad (19)$$

Where, $d_i = \sum_{j=1}^{n_i} \delta_{ij}$ is the number of events in the i^{th} clusters & $L_q(.)$ is the q^{th} derivative of LT for the FD of Z.

$$L^q(s) = -1^q \int_0^\infty Z^q \exp(-Zs) f(Z) dz;\ Where,\ q > 0. \quad (20)$$

Where, φ =vector of the parameters for a baseline HF, β =vector of the regression coefficients & θ =variance of a random effect. Then, $\varphi, \beta\ \&\ \theta$ were obtained by maximizing a marginal log-likelihood. $L^q(.)$ =LT up-to q=max $(d_1, ..., d_s).$

Prediction of Frailties

The frailty term Z_i was predicted as $Z_i = E[Z/z_i, \varphi, \beta, \theta],$ with z_i is the data of the i^{th} cluster. Conditional expectation [21]:

$$Z_i = E[Z/z_i, \varphi, \beta, \theta] = -\frac{L^{(d_i+1)}[\sum_{j=1}^{n_i} H_0(y_{ij})\exp(X_{ij}^T\beta)]}{L^{d_i}[\sum_{j=1}^{n_i} H_0(y_{ij})\exp(X_{ij}^T\beta)]} \quad (21)$$

Models Selection

Akaike Information Criterion (AIC): The model with the smallest AIC value was considered as a better fit [21].

Model Diagnostics

Likelihood Ratio Test (LRT): To test an association of covariates with an outcome in a frailty model.

Cox Snell Residuals: In semi-parametric residual plots were made with a redefinition of the various residuals to incorporate the parametric form of the baseline hazard rates [22].

Data quality control

Before data collection, an ethical approval was obtained from relevant ethics committees of the respective hospitals, was accessed and used a stroke patient datasets. Finally, data quality was ensured by implemented rigorous data collection procedures, including training for data collectors and regular data quality checks for stroke patients.

Result

Descriptive statistics

In this study, 224 charts were assessed for followed a stroke treatment in Harar General Hospital, Jegol Hospital, and Hiwot Fana Specialized University from September 1, 2020 to November 1, 2023. The main objective of this finding was to model the determinants of the time to death of stroke patients. From a total of 224 stroke patients, 51(22.78%) were dead and the rest, 173(77.23%), were censored. This is indicated by the fact that most off the stroke patients were cured after getting a different treatment in the hospitals. The hypertension disease in developing a stroke patients out of 224, 140 (62.5%) were absent of hypertension and the remaining 84 (37.5%) were present with hypertension. The death rate appears to be highest in stroke patients who had hypertension, 52.94%, compared to 47.06% in non-hypertensive stroke patients **(Table 2).**

When accounting for the 224 hospitals that received medical care, the percentages were 35.27%, 22.32%, and 42.41%, the hospitals that were treated in that order being Harar General Hospital, Jegol Hospital and Hiwot Fana Specialized University Hospital, respectively. According to the hospital mortality total, the rates at Harar General Hospital, Jegol Hospital, and Hiwot Fana Specialized University were 27.45%, 39.21%, and 33.34%, respectively. Similarly, fashion other variables were presented as detailed in table 2.

In table 3 shows that the mean age of the stroke patients was 55.7 years, with the oldest and youngest being 93 and 20 years old, respectively and with a standard deviation of 16.4. Out of 224 total stroke patents of the median survival time was 14 days for this study.

Survival of Significantly Different Groups

The log-rank test results in table 4 indicate that a significant difference in death events across the groups of hypertension, diabetes mellitus, atrial fibrillation, cardiac disease, and basic complications at the 5% level of significance.

The Kaplan-Meier (KM) estimator survival curve was used to estimate the survival function among different covariates so that one can make a comparison. Separate graphs of the estimates of the KM survivor functions were constructed for different categorical covariates. In general, the survivorship pattern of one was laid above another, which means that the group was defined by the upper curve has a better survival rate than the group was defined by the lower curve (Figure 1).

Table 2: Descriptive statistics for categorical variables of stroke patients from September 1, 2020 to November 1, 2023 in Harari regional state.

Covariate Variables	Category	Patients Status		
		Censored	Dead	Total
Sex	Female	60(75%)	20(25%)	80(35.71%)
	Male	113(78.47%)	31(21.53%)	144(64.29%)
Residence	Urban	81(75%)	27(25%)	108(48.21%)
	Rural	92(79.31)	24(20.69%)	116(51.79%)
Hypertension	No	116(82.86)	24(17.14%)	140(62.50%)
	Yes	57(67.86%)	27(32.14%)	84(37.50%)
Cardiac Disease	No	129(84.87%)	23(15.13%)	152(67.86%)
	Yes	44(61.11%)	28(38.89%)	72(32.14%)
Diabetes Mellitus	No	148(86.05%)	24(13.95%)	172(76.79%)
	Yes	25(48.08)	27(51.92%)	52(23.21%)
Atrial fibrillation	No	151(86.29%)	24(13.71%)	175(78.12%)
	Yes	22(44.90)	27(51.10%)	49(21.88)
Basic complication	No	108(83.72%)	21(16.28%)	129(57.59%)
	Yes	65(68.42%)	30(31.58%)	95(42.41%)
Stroke types	Ischemic	114(77.03%)	34(22.97%)	148(66.07%)
	Hemorrhagic	59(77.63%)	17(22.37%)	76(33.93%)
Drug types	Anti-coagulants and Anti-platelet	12(66.67%)	6(33.33%)	18(8.04%)
	Anti-coagulants, anti-platelet and anti-hypertensive	19(82.61%)	4(17.39)	23(10.27%)
	Anti-coagulants, anti-platelet, and statin	28(84.85%)	5(15.15%)	33(14.73%)
	Anti-coagulants, anti-platelet, statin and anti-hypertensive	25(73.53%)	9(26.47%)	34(15.18%)
	Anti-coagulants, anti-platelet, statin, anti-hypertensive and antibiotics	17(70.83%)	7(29.17)	24(10.71%)
	Anti-platelet, statin and anti-hypertensive	7(77.78%)	2(22.22%)	9(4.02%)
	Anti-hypertensive and antibiotics	18(78.26%)	5(21.74)	23(10.27%)
	Anti-coagulants and anti-hypertensive	15(88.24%)	2(11.76%)	17(7.59%)
	Other	32(74.42%)	11(25.58%)	43(19.20%)
Hospitals	Harar General Hospital	65(82.28%)	14(17.72%)	79(35.27%)
	Jegol Hospital	30(60%)	20(40%)	50(22.32%)
	Hiwot Fana Specialized University Hospital	78(82.11%)	17(17.89%)	95(42.41%)

Table 3: Descriptive statistics for continuous variables of stroke patients

Age	**Minimum**	**Maximum**	**Mean**	**Standard deviation**
	20	93	55.71	16.39
Survival time	**n**	**Events**	**Median**	**95% CI**
	224	51	14	[10, NA]

Table 4: Log rank test for equality of survival function of different groups

Covariates	Chi-square value	df	Pr > Chi-Square
Sex	0	1	0.8
Residence	0.1	1	0.7
Hypertension	3.5	1	0.045
Cardiac Disease	12.9	1	<0.001
Diabetes Mellitus	19.9	1	<0.001
Atrial fibrillation	37.5	1	<0.001
Basic complication	4.3	1	0.04
Stroke types	0.1	1	0.8
Drug types	6.4	8	0.6

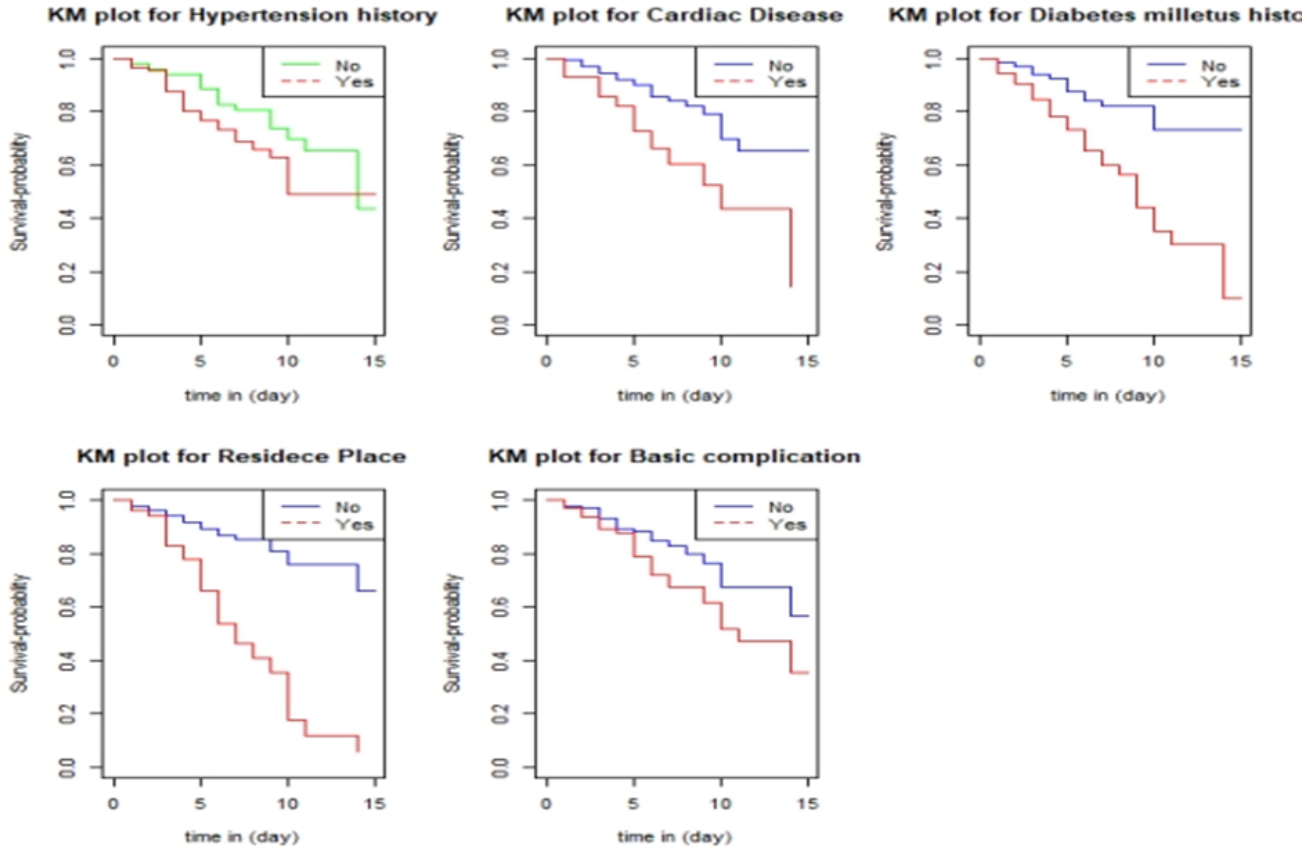

Figure 1: *Kaplan-Meier survivor curves for significantly difference groups*

Test Unobserved Heterogeneity

To predict the random effect of θ to get an idea of heterogeneity among clusters. When θ is large and significant, indicates that a heterogeneity among clusters and a strong correlation among individuals in the same cluster. Conversely, when θ=0, the frailties=1, suggesting that there were no-clustered effects and that events were occurred independently for both in-side and between clusters [15]. The outcomes given in Table 3.4 shows that the likelihood ratio tests of variance of random term(θ) for exponential gamma, exponential inverse Gaussian, Weibull gamma, Weibull inverse Gaussian, log-logistic gamma, and log-logistic inverse Gaussian shared frailty models whose p<0.001 for all shared frailty models. Thus, refers that an indications of unobservable heterogeneity was significant effect (P<0.05) for all models in the stroke patient datasets.

The variance of random effect was highest (θ=0.82) for the Weibull inverse Gaussian shared frailty model and the least (θ=0.23) for the exponential gamma shared frailty model with an exponential baseline hazard. A Kendall's tau(τ) was used to measure the dependence with-in the hospitals (clusters). The values of τ for exponential gamma, exponential inverse

Gaussian, Weibull gamma, Weibull inverse Gaussian, log-logistic gamma, log-logistic inverse Gaussian, log-normal gamma, and log-normal inverse Gaussian shared frailty models were 0.101, 0.097, 0.151, 0.201, 0.149, 0.189, 0.135, and 0.123, respectively. This reveled that, on average had a positive correlation between time-to-death and stroke patients with-in the hospitals **(Table 5).**

Table 5: Test of unobserved heterogeneity using LRT

Shared Frailty Model	LRT	θ	τ	P- value
Exponential Gamma	38.02087	0.225	0.101	<0.001
Exponential Inverse Gaussian	38.25056	0.264	0.097	<0.001
Weibull Gamma	58.07973	0.356	0.151	<0.001
Weibull Inverse Gaussian	58.43818	0.822	0.201	<0.001
Log-logistic Gamma	57.68213	0.349	0.149	<0.001
Log-logistic Inverse Gaussian	58.03188	0.734	0.189	<0.001
Log-normal Gamma	55.79835	0.312	0.135	<0.001
Log-normal Inverse Gaussian	56.11336	0.367	0.123	<0.001

LRT=likelihood ratio test, θ=variance of random terms, τ=Kendall's tau

Model Comparison

In table 6 summarized that all the outcomes of the four baseline hazard functions with two frailty models. Among those models, the Inverse Gaussian frailty model with the Weibull baseline hazard function had the smallest AIC of 383.46. It was the most appropriate model to describe time-to-death for stroke patient datasets.

Table 6: AIC values for the parametric frailty models

Baseline Hazard Function	Frailty Distribution	AIC
Exponential	Gamma	401.877
	Inverse Gaussian	401.6473
Weibull	Gamma	383.8181
	Inverse Gaussian	383.4597
Loglogistic	Gamma	384.2157
	Inverse Gaussian	383.866
Lognormal	Gamma	384.2157
	Inverse Gaussian	385.7845

Multivariable Analysis

A findings of a multivariable frailty models were revealed that the covariates: hypertension(φ=2.118; 95%CI:1.145-3.917), cardiac disease(φ=2.667; 95%CI:1.343-5.296), diabetes mellitus(φ=3.035; 95%CI:1.1.560-5.906), atrial fibrillation(φ=3.247; 95%CI:1.619-6.511), and basic complications (φ=2.983; 95% CI:1.477-6.023) had a significant frailty effect(P<0.05) on the time to death of stroke patients. However, sex was the only insignificant effect(P>0.05) in the model. A variable sex had contained one in the 95% CI of an acceleration effect(φ) in the models in table 7 The interpretation for accelerated factor (φ) under the final multivariate shared frailty model for a stroke patients datasets were as followed: The value φ=2.12 represents a risk ratio. A risk ratio of 2.12 means that for every 100 people with hypertension experiencing the outcome, there would be approximately $47(100/2.12)=47$ people without hypertension experiencing the same outcome. In health terms: this finding highlights hypertension as a serious health concern. It underscores the importance of managing blood pressure to reduce the risk of these related complications. Effective management strategies, such as lifestyle changes and medication, can help mitigate the increased risk associated with hypertension.

A variable cardiac disease had a value of φ=2.67 represents a risk ratio. A risk ratio of 2.67 means that for every 100 people with cardiac disease experiencing in the stroke patients, there would be approximately 38 people without cardiac disease experiencing in the stroke patients. A risk ratio(φ)=2.67, indicates that for every 100 people with car-

diac disease had experienced in the stroke patients, approximately 38 people without cardiac disease had experienced in the stroke patients. A value of φ=3.04(risk ratio) means that for every 100 people with diabetes mellitus were experienced in the stroke patients, approximately 33 people without diabetes mellitus were experienced in the stroke patients. A risk ratio of 3.25(φ=3.25) revealed that for every 100 people with atrial fibrillation was experienced in the stroke patients, there would be approximately 31 people without atrial fibrillation was experienced in the stroke patients. Similarly, A basic complications had φ=2.98 =risk ratio, means that for every 1000 people with basic complications were experienced in the stroke patients, that leads approximately 34 people without basic complications experiencing in the stroke patients.

Table 7: Weibull versus inverse Gaussian multivariable analysis shared frailty model

Covariates	Category	β	φ	St. err	P-value	95% CI
Sex [Female(Ref.)]	Male	0.167	1.181252	0.326	0.726	[0.624, 2.237]
Hypertension [No(Ref.)]	Yes	0.75	2.117636	0.314	0.013 *	[1.145, 3.917]*
Cardiac Disease [No(Ref.)]	Yes	0.981	2.667516	0.35	0.004 **	[1.343, 5.296]*
Diabetes Mellitus [No(Ref.)]	Yes	1.11	3.035117	0.34	0.001 **	[1.560, 5.906]*
Atrial fibrillation [No(Ref.)]	Yes	1.178	3.246609	0.355	0.002 **	[1.619, 6.511]*
Basic complication [No(Ref.)]	Yes	1.093	2.983047	0.359	0.003 **	[1.477, 6.023]*

τ=0.201; θ=0.822; λ=0.014; ρ=1.667; AIC= 383.4597.

φ=acceleration factor, τ=Kendall's tau, θ=variance of random effect, λ=scale, ρ=shape, CI=Confidence Interval & Ref.= reference.

Checking for Overall Goodness of Fit

Diagnostic Plots of the Parametric Baselines

To ascertain if a fitted parametric model accurately represents the data, this choice needs to be made. Out of the four parametric baseline graphs, the Weibull curve exhibits greater linearity than others. This suggests that a Weibull baseline hazard is a better choice for the stroke disease datasets **(Figure 2).**

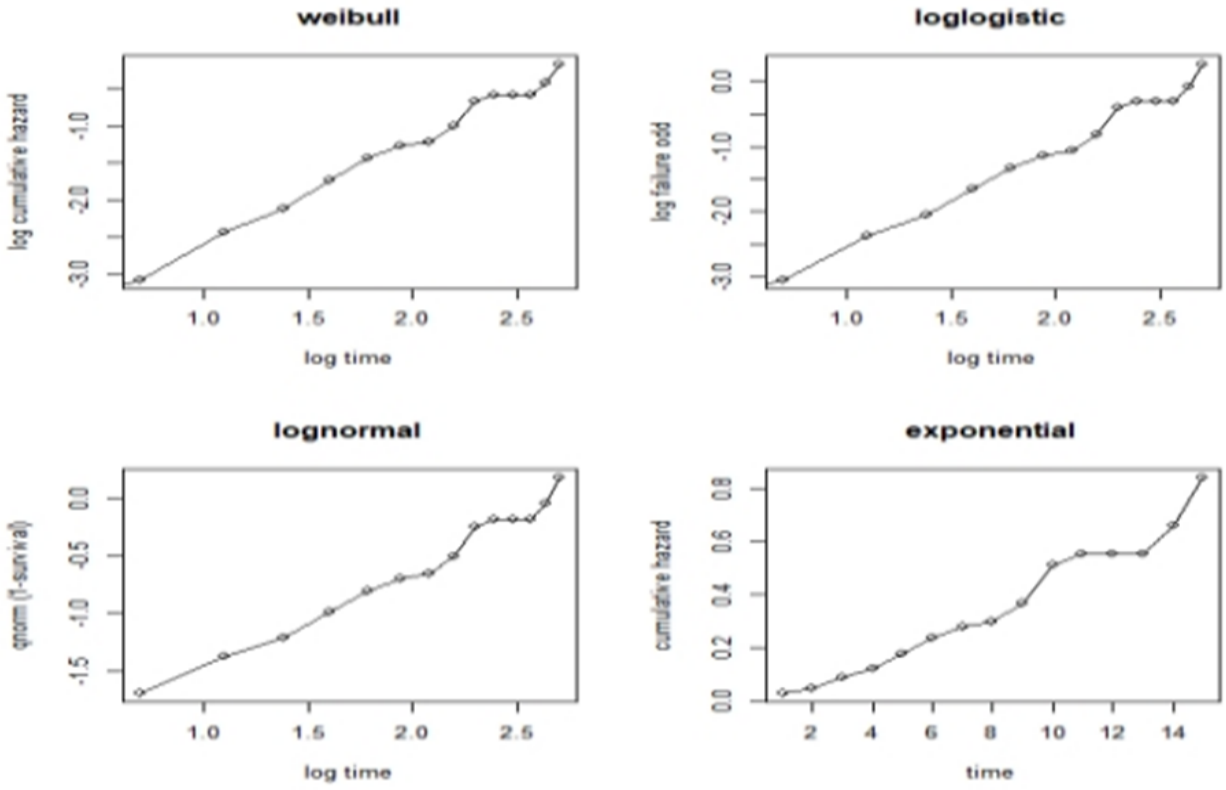

Figure 2: *Diagnostic plot for baselines hazards*

Cox Snell Residual Plots

The residual plot for the Weibull hazard function is rather near the 45-degree straight line through the origin. The **figure 3** revealed that out of all models, the Weibull model that was fitted well to the data was accepted.

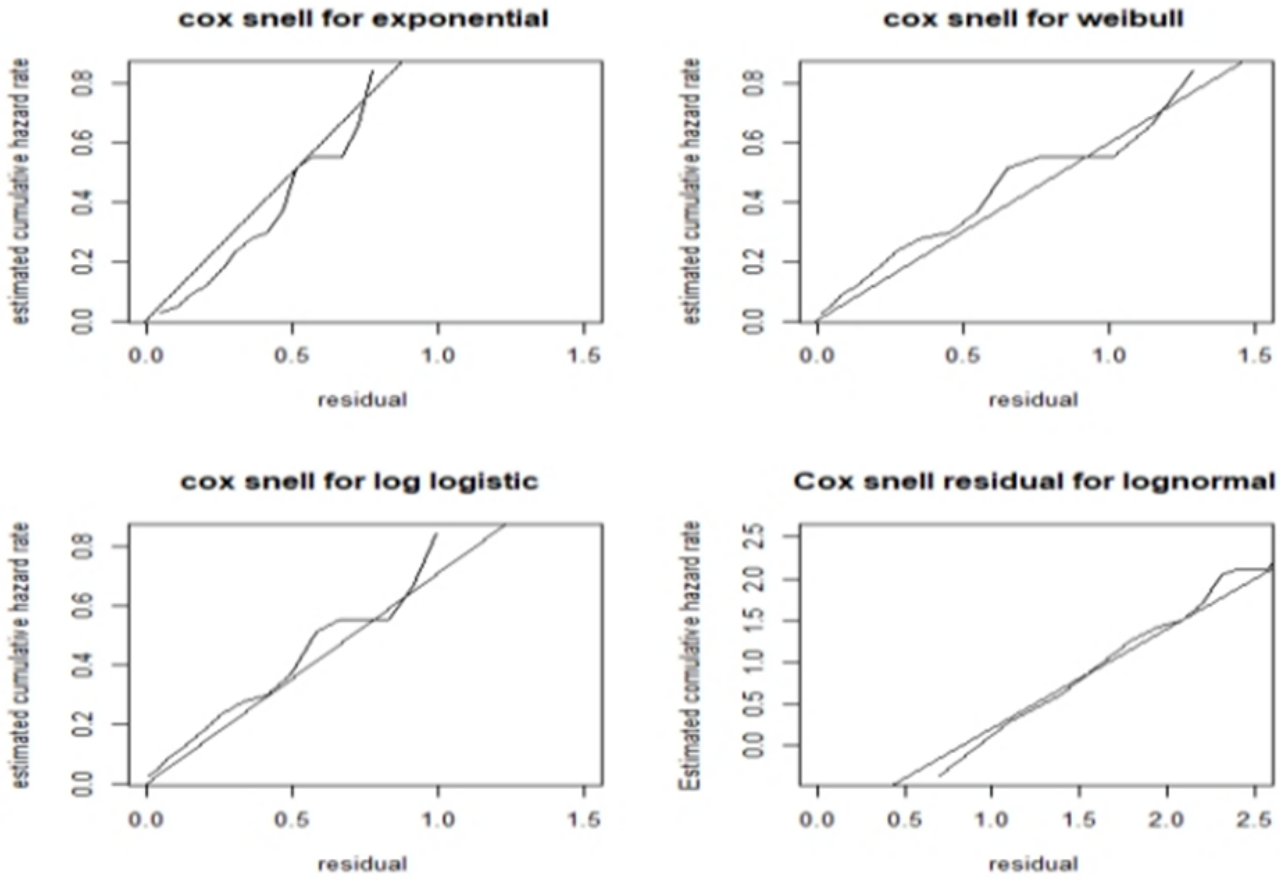

Figure 3: *Cox snell residual plots*

Discussion

This study aimed to model a parametric shared frailty model. The time to death of stroke patients data were used for gamma and inverse-Gaussian frailty distributions among the various baseline hazard functions, including exponential, Weibull, log-logistic, and lognormal. In this study, the population in the same hospital relatively shared some factors, such as the skill of doctors, bedrooms, environment, health facilities, and resources in determining the time to death of stroke patients. A findings of a multivariate shared frailty model revealed that hypertension, cardiac disease, diabetes mellitus, atrial fibrillation, and basic complications had a significant frailty effect (P<0/05) on the stroke patients. However, the variable sex was the only insignificant frailty effect (P>0.05) on the stroke patients. A Weibull inverse Gaussian frailty model had the smallest AIC of 383.46, which makes it the most suitable for explaining time-to-death in the stroke patient datasets. These study findings were supported with stroke patients in Africa by Akinyemi R. et. al., 2019[23].

An outcomes of the models showed that hypertension had a significant impact on the time to death of stroke patients. The prognostic factor of hypertension has increased the risk by a factor of φ=2.12 was compared to patients with non-hypertension; other covariates have remained constant. The value φ=2.12 represents a risk ratio. A risk ratio of 2.12 means that for every 100 people with hypertension experiencing the outcome, there would be approximately $47(100/2.12)=47$ people without hypertension experiencing the same outcome. In health terms: this finding highlights hypertension as a serious health concern. It underscores the importance of managing blood pressure to reduce the risk of these related complications. Effective

management strategies, such as lifestyle changes and medication, can help mitigate the increased risk associated with hypertension.

The results were compared with a related study was conducted at Felege Hiwot Referral Hospital using a retrospective cohort design by Abay Kassie et. al., 2019[24]. The time to death for stroke patients was significantly impacted by cardiac disease, depending on the prognostic factors. For patients without cardiac disease, the model's output shows that the prognostic factor for cardiac disease has increased by a factor of φ=2.67, which was in line with study conducted in Saudi Arabia by Alhazzani A. et. al., 2018[25]. A risk ratio (φ)=2.67, indicates that for every 100 people with cardiac disease had experienced in the stroke patients, approximately 38 people without cardiac disease had experienced in the stroke patients.

According to this study a diabetes mellitus to be a significant risk factor for stroke patients' death, and other studies have continuously corroborated with the study was conducted by Amanual G. et. al., 2019 [26]. The results of the Weibull-inverse Gaussian frailty model showed that diabetes mellitus, which increases the risk of death by a factor of φ=3.034, compared to the reference groups. A value of φ=3.04(risk ratio) means that for every 100 people with diabetes mellitus were experienced in the stroke patients, approximately 33 people without diabetes mellitus were experienced in the stroke patients. Atrial fibrillation was a significant on the time to death of stroke patients at a 5% level of significance. A risk ratio of 3.25(φ=3.25) revealed that for every 100 people with atrial fibrillation was experienced in the stroke patients, there would be approximately 31 people without atrial fibrillation was experienced in the stroke patients. The similarity study was done at Addis Ababa, Ethiopia using a retrospective study design by Ayehu K., et. al., 2020 [27].

When compared to the reference groups, the basic complication increased the risk of mortality by a factor of φ=2.98, which had a significant impact on the survival time of stroke patients at a 5% level of significance. A basic complications had φ=2.98 =risk ratio, means that for every 1000 people with basic complications were experienced in the stroke patients, that leads approximately 34 people without basic complications experiencing in the stroke patients. A related study was carried out at Mettu Karal Referal Hosipital by Dereje G. and Azmeraw G., 2022[28].

The Weibull baseline was the best fit for the stroke datasets, when compared to the exponential, log-logistic, and log-normal hazard functions. Diagnostic graphs were generated to evaluate the model's suitability. The Weibull plot of log cumulative hazard versus log time was more linear. This findings were further supported by the cumulative hazard plot for the Cox snell residuals of the log-normal, Weibull, exponential, and log-logistic models. In this case the plots were closer to the line, shows that the Weibull model performed the best, the synonyms study was conducted at New York by Klein J. and Moeschberger M., 2003[29].

Limitations: Due to the lack of proper stroke patients' data management at those hospitals, some of the vital factors were not included, such as family history, heavy alcohol consumption, physical inactivity, and smoking status.

Conclusion

This study highlights the importance of the hospital-level cluster effects in the survival analysis of stroke patients. The significant frailty effect suggests variability in outcomes across hospitals, underlining the need for tailored interventions. Clinicians and hospital administrators should consider these differences when managing stroke patients, emphasizing timely follow-up, individualized care, and resource allocation to improve the survival outcomes.

List of Abbreviations: SSA: Sub-Saharan African; WHO: World Health Organization; ST: Survival Time; PDF: Probability Distribution Function; CDF: Cumulative Distribution Function; SF: Survival Function; HF: Hazard Function; KME: Kaplan-Meier Estimator; MST: Median Survival Time; MF: Modeling Frailty; SFM: Shared Frailty Model; CHF: Cumulative Hazard Function; PHFM: Proportional Hazard Frailty Model; AFTFM: Accelerated Failure Time Frailty Model; GFD: Gamma Frailty Distribution; IGFD: Inverse Gaussian Frailty Distribution; AIC: Akaike Information Criterion; LRT: Likelihood Ratio Test.

Declarations

Ethical Approval and Consent to Participate: Ethical clearance had been obtained from Department of Statistics, Haramaya University, Ethiopia #5487, issued on October 03, 2023.

Availability of Data and Materials: This work is basically considered the Harari health office to collect stroke patient data from three hospitals such as Harar General Hospital, Jegol Hospital and Hiwot Fana Specialized University Hospital determinates were included under this study.

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The authors acknowledge Harari health office was permitted to collect stroke patient data from these three hospitals.

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Author Contributions: KA is developed the original draft preparation, conceptualization, data collection, analysis & interpretation; AA prepared a manuscript and report writing and KT reviewed and edited the overall document.

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Prevalence and associated factors of periodontal disease in Ethiopia: systematic review and meta-analysis

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Abstract

Background: *Periodontal disease, a chronic bacterial infection of the periodontium, is the major cause of tooth loss, masticatory dysfunction, and edentulism. These outcomes adversely affect nutritional status, self-esteem, and quality of life, while also contributing to significant healthcare costs and socio-economic burden. Although oral diseases affect approximately 3.5 billion people globally, with 75% residing in low- and middle -income countries, the national prevalence of periodontal disease in Ethiopia remains undetermined.*

Objective: *This review aimed to determine the pooled prevalence and associated factors of periodontal disease in Ethiopia.*

Method*: A systematic search was conducted across PubMed, Scopus, Embase, and Google Scholar to identify relevant studies on periodontal disease in Ethiopia, from inception to April 2023, with no language restrictions. Data were extracted using Microsoft Excel and analyzed in Stata version 17. The pooled prevalence and associated factors were estimated using a random-effects mode due to the high heterogeneity. Heterogeneity was assessed using the Higgs I^2 statistic and the Cochrane Q test. Subgroup and sensitivity analyses were conducted to explore source of heterogeneity. The review adhered to the PRISMA guidelines, and the protocol was registered in PROSPERO (CRD42023415994).*

Result: *Thirteen studies comprising 10,744 participants were included. The pooled prevalence of periodontal disease in Ethiopia was 35% (95% CI: 25% – 45%) with substantial heterogeneity (I^2=99.46%). The highest prevalence was reported in Addis Ababa (42%; 95% CI: 29% to 55%) and among institutionalized individuals (49%; 95% CI: 44 – 55%). Regular toothbrushing was associated with lower odds of periodontal disease (OR = 0.26; 95% CI: 0.24 – 0.28), while male gender (OR = 0.72, 95% CI: 0.64 – 0.80) and carbohydrate intake (OR = 0.54, 95% CI: 0.44 – 0.65) were significantly associated with a reduced risk of periodontal disease in Ethiopia.*

Conclusion: *Periodontal disease affects approximately one in three Ethiopians, with higher prevalence among institutionalized populations. Key determinants include inadequate oral hygiene, female gender, and dietary habits. These findings underscore the need for targeted oral health interventions, including the promotion of regular tooth brushing and dietary modification. National policies should support standardized diagnostic guidelines and integrate oral health services into broader community health programs.*

Introduction

Periodontal disease is a chronic inflammatory condition of the periodontium, leading to the progressive destruction of the periodontal ligament and surrounding alveolar bone (1, 2). According to the 2022 World Health Organization (WHO) Global Oral Health Status Report, around 3.5 billion people worldwide are affected by oral diseases, disproportionately affecting populations in low- and middle-income countries (LMICs) (75%) (3). In Ethiopia, the burden of periodontal disease is likely substantial because of poor dental services coverage and high unmet needs. Yet, it remains poorly documented due to the absence of a national surveillance system.

The Global Burden of Disease (GBD) study ranks periodontal disease as the 11th most prevalent condition globally, with prevalence ranging from 20% to 50% (4). A meta-analysis from India found that 51% of adults had periodontal disease (5), while data from the USA indicated that 47% of the adults suffer from moderate-to-severe forms of the condition (6).

Periodontal disease is the leading cause of tooth loss, posing significant public health challenges (7, 8). In its advanced stages, periodontitis contributes to multiple tooth loss, masticatory dysfunction, and edentulism, which negatively impact nutrition, self-esteem, social functioning, and quality of life, while also imposing huge socio-economic and healthcare costs (9-11). The oral cavity harbors over 800 species of bacteria, and periodontal disease arises from complex interactions between microbial biofilm and the host immune response. Poor oral health behaviors, including smoking and inadequate tooth brushing habits, often exacerbate this interaction (7, 8). Besides these behavioral factors, sociodemographic and psychosocial factors such as low income, limited education, advanced age (2, 12, 13) and psychosocial stress has been implicated as a risk factor for periodontal disease(14).

Emerging evidence highlights a bidirectional relationship between periodontal disease and several health conditions. The systemic dissemination of periodontal pathogens and inflammatory mediators has been associated with a range of systemic diseases or conditions(15), including cardiovascular disease, adverse pregnancy outcomes, cancer, respiratory diseases, diabetes mellitus, and chronic kidney disease(16-19). The biological plausibility of these associations is primarily attributed to the systemic inflammatory burden generated by periodontitis (16-19).

Despite being a largely preventable and manageable condition, the global prevalence of periodontitis is increasing. This underscores the need for enhanced strategies for early detection, prevention, and treatment. The global prevalence of severe periodontal disease is gradually increasing despite efforts to prevent and control the progression of periodontitis (20). In response, there has been a global call since 2017 to integrate periodontal care into the health systems and public health frameworks (21).

Ethiopia, a low-income country in East Africa with an estimated population of 120 million, lacks a national surveillance system for oral health, including periodontal disease. Existing data from isolated cross-sectional studies show a wide variation in prevalence, from 7% (22) to 75.8% (23), highlighting both the magnitude of the problem and the inconsistency in reporting. To date, there has been no comprehensive synthesis of the available evidence on the prevalence of periodontal disease and its associated factors in Ethiopia.

Therefore, this systematic review and meta-analysis was conducted to fill this knowledge gap. Specifically, it aims to estimate the pooled prevalence of periodontal disease in Ethiopia and identify its key associated factors. The findings address a critical knowledge gap and provide evidence to inform national oral health planning, prevention strategies, and policy integration with Ethiopia's broader health system.

Research Question

Among the Ethiopian population, what is the prevalence of periodontal disease, and what factors are associated with an increased risk?

Method

Study Design and Protocol Registration

This systematic review and meta-analysis was conducted following the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) checklist (24). We registered the protocol in the International Prospective Register of Systematic Reviews (PROSPERO) under registration ID PROSPERO database (CRD42023415994).

Information Sources and Search Strategy

We conducted a systematic search of studies using electronic databases (PubMed, Scopus, and Embase) and websites (Google and Google Scholar) covering all publications from the inception to March 2023. Search terms including combinations of Medical Subject Headings (MeSH) and keywords such as "periodontal disease" OR "Periodontitis" OR "oral disease" OR "gum disease" AND "Prevalence" OR "epidemiology" AND "determinants" OR "risk factor" AND "Ethiopia". Additionally, reference lists of included articles were manually screened to identify any relevant studies missed during the database search. We compiled the search results using EndNote reference citation management software. The article search was done from April 05 to 10/2023. No restrictions were placed on the language of publication during the search.

Eligibility criteria

Studies were included if they: (1) were conducted in Ethiopia; (2) were population/community level, institutional, or hospital-based studies; (3) included human participants; (4) with a clear definition and measurement of periodontitis; and (5) reported prevalence data or had sufficient information to estimate the prevalence.

Excluded studies were: (1) studies focused on one gender only (i.e., pregnancy-related periodontal disease); (2) conducted on animals; (3) not reporting prevalence or associated factors; or (4) were reviews, case reports, or editorials.

Study selection

All studies retrieved during the article search was imported into EndNote 20 for reference management, and duplicate records were removed. The screening process for article selection was completed in two stages. Initially, the titles and abstracts of all identified studies were screened independently by two reviewers (AT & SS) to assess eligibility based on the inclusion and exclusion criteria. In the second stage, full-text articles of potentially relevant studies were retrieved and reviewed in detail to determine final inclusion. Any disagreement between the two reviewers was resolved through discussion. When consensus could not be reached, a third investigator (NB) was consulted to make the final decision.

Data extraction

The entire potentially relevant information from the included studies was independently extracted by two investigators (AT & NB) using a pre-designed, standardized Microsoft Excel form. The data extracted includes the following variables: name of primary author, year of publication, study region, sample size, study population (institution-based or community-based), study setting (community, school, or institution), prevalence of periodontal disease, diagnostic tool of periodontitis, and possible predictor variables. In case of disagreement between the two reviewers, a third investigator (MS) was consulted to reach a consensus and made the final decision. For predictor variable assessment, we extracted effect size estimates such as odds ratios (ORs) along with their corresponding 95% confidence intervals (CIs) for each included study.

Risk of bias assessment

The quality of the included articles was evaluated using a 10 -item critical appraisal tool adapted from Hoy et al.'s risk of bias tool for prevalence studies (25). The tool consists of 10 items, of which the first four items assessed the externa validity of the study (domains are section and non-response bias), and items 5 to 10 assessed the internal validity of the study (items 5 to 9 assess the domain of measurement bias, and item 10 assesses bias related to analysis). Each study received a score ranging from 0 to 10, with a lower score indicating a higher risk of bias. Based on total score, the overall risk of bias was categorized as low risk of bias (7-10), moderate risk of bias (4-6), and high risk of bias (0-3) scores. Two reviewers (DA and SS) independently assessed the quality of each study, and they resolved any discrepancies by consulting a third reviewer (MS).

Outcome of interest

The primary outcome of this review was the pooled prevalence of periodontal disease among the Ethiopian population. The secondary outcome was the identification and synthesis of predictor variables associated with periodontal diseases reported across the included studies.

Statistical analysis and synthesis of findings

Before conducting the pooled analysis, we evaluated the presence of heterogeneity among the included studies I^2 test (Higgins' method) and Cochrane's Q test. An I^2 value greater than 75% combined with a statistically significant P-value ($P<0.05$) was considered indicative of high heterogeneity. In such cases, a random effect model was used. If heterogeny was low (or had a non-significant P-value a fixed effects model was applied.

The meta-analysis was conducted using STATA version 17 software. The pooled prevalence of periodontal disease was calculated with a 95% confidence interval (CI), based on sample size and number of periodontal patients. To explore the source of heterogeneity, subgroup analysis was done based on region and type of population. Moreover, a sensitivity analysis was done to examine the presence of potential influential studies of the pooled prevalence by sequentially excluding individual studies to identify the potential outliers or overinfluential studies. Publication bias was assessed using visual inspection of the funnel plot, Egger's regression test, and Doi plots. The Doi plots, accompanied by the Luis Furuya-Kanamori (LFK) index, is a quantitative measure of asymmetry in the Doi plots. An LFK index value within ±1 indicates no asymmetry, values between ±1 and ±2 suggest minor asymmetry, and values beyond ±2 indicate major asymmetry. Where publication bias was detected, the trim and fill method was applied to estimate and adjust for the potential impact of missing studies on the pooled prevalence estimate.

Predictor variables were analyzed using a random-effects meta-analysis model. Adjusted odds ratios (AORs) and their corresponding standard errors were log-transformed and pooled using the generic inverse variance method. Separate meta-analyses were conducted for each predictor reported in at least two studies.

Result

Selection of Included Studies

A total of 101 articles were retrieved electronically using databases and gray literature sources. Of these, 21 were duplicates and were removed using EndNote 20. The remaining 80 articles were screened based on titles and abstracts. Of these, 53 articles were found to be irrelevant to the study objectives and were excluded. The full texts of the remaining 27 articles were then assessed against the inclusion and exclusion criteria. Following the full-text review, 13 articles met all eligibility criteria and were included in the final systematic review and meta-analysis. A detailed summary of the screening and selection process is illustrated in the PRISMA flow diagram (**Figure 1).**

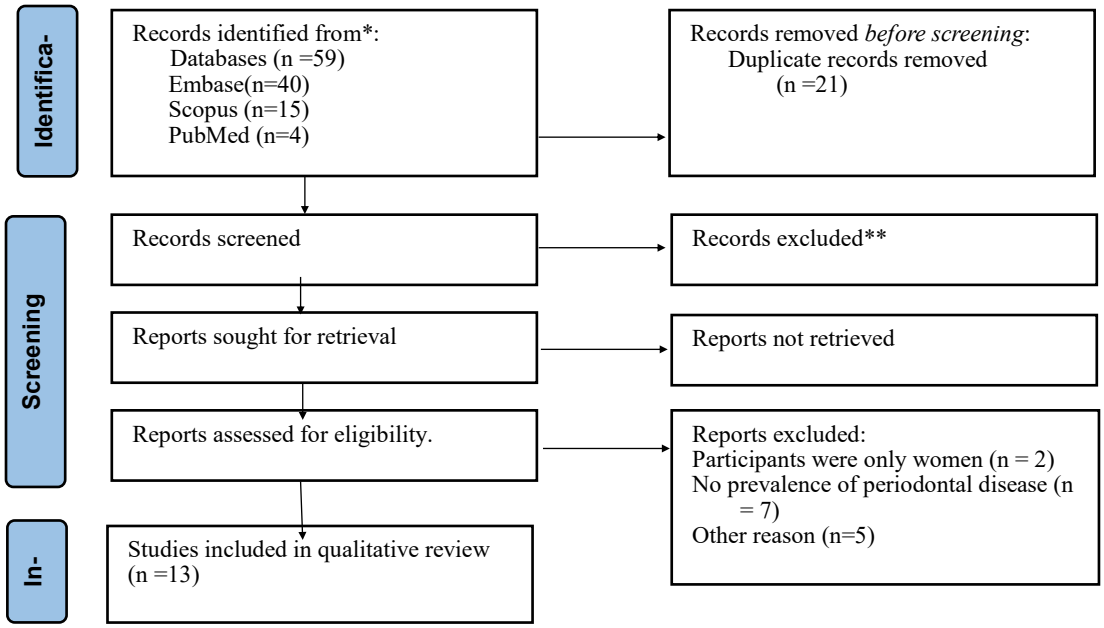

Figure 1: *PRISMA flow diagram showing the article selection process*

Characteristics of the included studies

The included 13 cross-sectional studies were conducted in three regions of Ethiopia. Five of the included studies were conducted in Addis Ababa, followed by Amhara (n=4) and Oromia (n=4). The study settings varied, including community-based studies (n=4), hospital-based studies (n=4), and institutional settings such as schools, prisons, and care homes (n=5). Of these, one study specifically targeted students living with disabilities. The sample size ranged from 132 (26) to 3,451(27) participants, with a total pooled sample of 10,744 individuals. Of these, 5,479 (50.99%) participants were male. The included studies were conducted from 1978 to 2022. The reported prevalence of periodontal disease across studies ranged from 7.0% (22) to 75.83% (23) (**Table 1**).

Table 1: Characteristics of the included studies

S. No	Author (year)	Place of the study	Study setting	Sample size	Prevalence	Risk of bias
	Vered et al., 2003 (28)	Amhara	Community-based study	487	0.1396	Low
	Zini et al., 2009(29)	Amhara	Community-based study	340	0.3647	Low
	Diro et al., 2008(30)	Addis Ababa	Hospital-based study	384	0.2317	Moderate
	Meyrema & Kedir, 2018(31)	Oromia	School-based study	422	0.4384	Low
	Duguma et al., 2019(32)	Addis Ababa	Institutionalized	182	0.5109	Moderate
	Derara et al., 2021(27)	Oromia	Hospital-based study	3,451	0.1083	Moderate
	Burnet et a, 2015(26)	Addis Ababa	Institutionalized	132	0.4696	Moderate
	Simon et al, 2003(33)	Addis Ababa	school based	1736	0.5339	Low
	Kebede et al, 2012(23)	Oromia	Hospital based	240	0.7583	Low
	Berhane & Worku, 2014(34)	Addis Ababa	Community based	658	0.3541	Low
	Olsson, 1978(22)	Oromia	Community based	1,700	0.07	low
	Tefera &Bekele, 2020(35)	Amhara	Hospital based	420	0.2761	Low
	Tefera et a, 2022l(36)	Amhara	School-based (disabled students)	443	0.2753	Low

Prevalence of periodontal disease

The overall pooled prevalence of periodontal disease in Ethiopia was found to be 35% (95% CI: 25-45). A high degree of heterogeneity was observed among studies (I^2=99.46%, P <0.001), indicating substantial variability between the included studies. Given this heterogeneity, a random-effect model was applied for the meta-analysis. Each square in the forest plot represents the prevalence estimate from an individual study, with the size proportional to the study's weight in the meta-analysis. Horizontal lines indicate 95% confidence intervals. The vertical line represents the overall pooled prevalence. The diamond at the bottom shows the pooled prevalence estimate with its 95% confidence interval using a random-effects model **(Figure 2).**

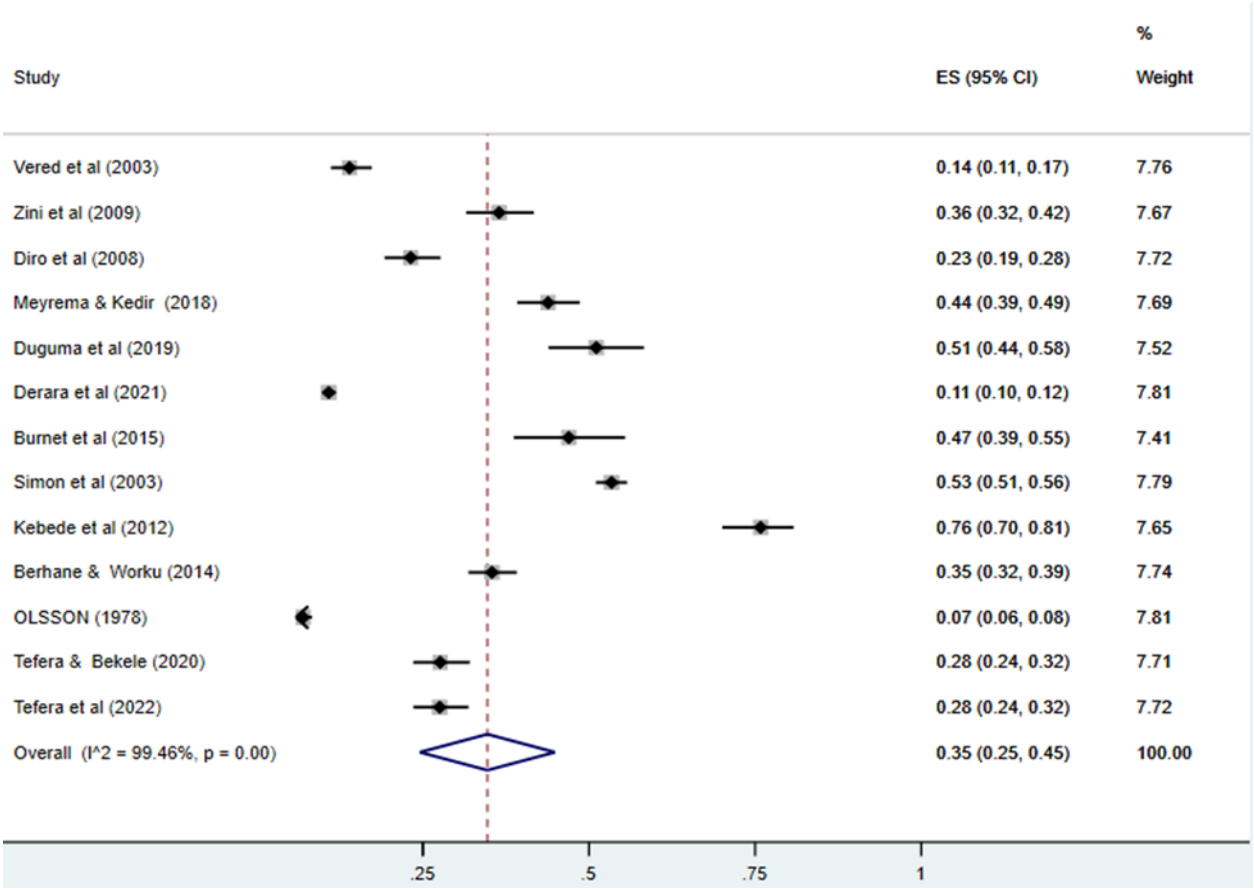

***Figure 2**: Forest plot of pooled prevalence estimates of periodontal disease in Ethiopia.*

Subgroup analysis based on region

To explore the source of heterogeneity and determine regional variation in the prevalence of periodontal disease, a subgroup analysis was done by region. The analysis revealed that the highest pooled prevalence was observed in Addis Ababa, at 42% (95% CI: 29% to 55%), while the lowest prevalence was reported in the Amhara region, at 26% (95% CI: 17% to 36%) **(Figure 3).**

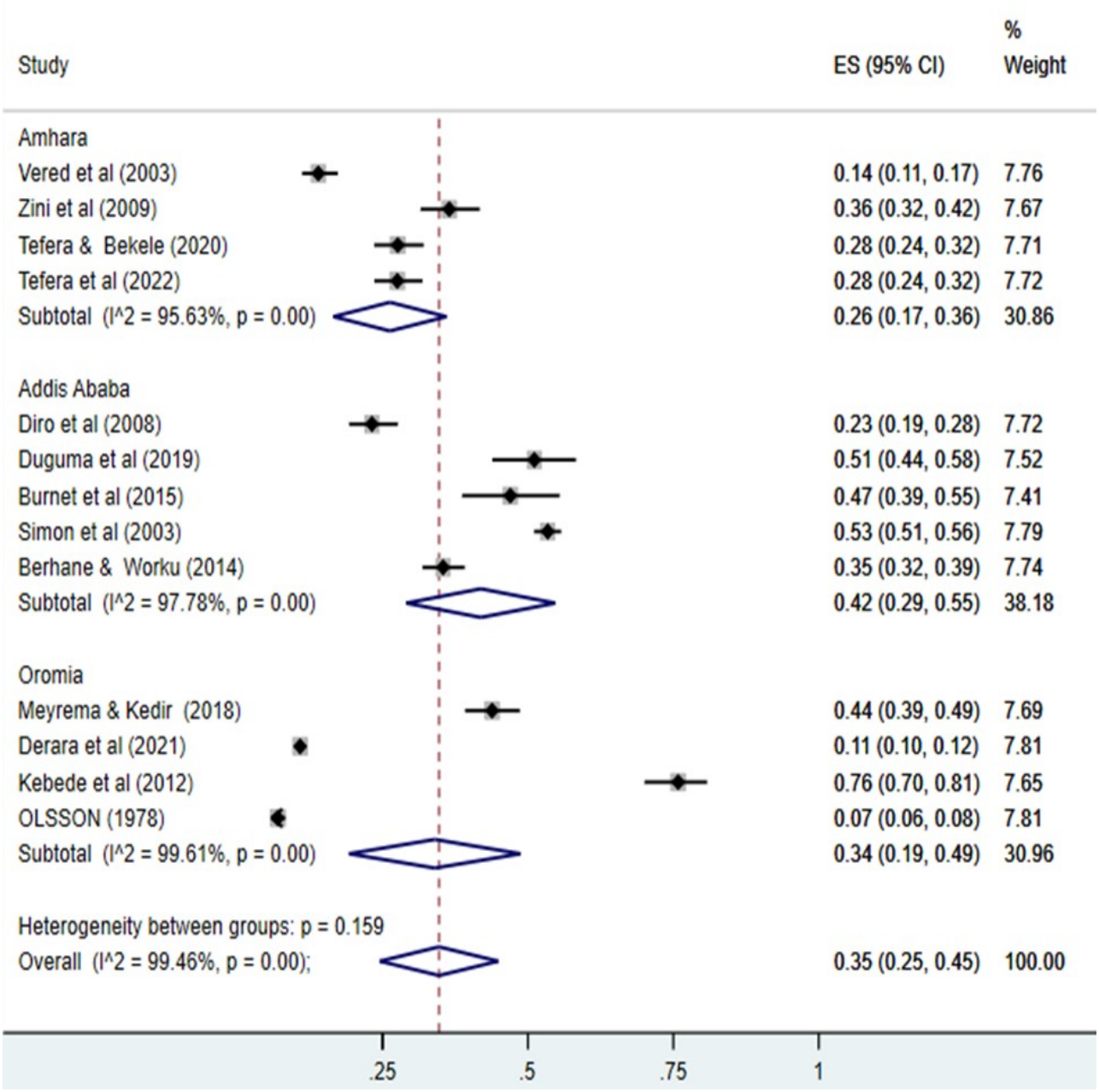

Figure 3: *Subgroup analysis of periodontal disease based on region*

Subgroup analysis was also conducted based on population type to identify differences in the prevalence of periodontal disease across various groups. The highest pooled prevalence was observed among students, at 52% (95% CI: 49% to 54%), followed by institutionalized individuals, with a prevalence of 49%. These results are illustrated in **Figure 4.**

Sensitivity analysis

Sensitivity analysis was conducted by systematically excluding one study at a time (leave-one-out method) to determine the influence of each study on the pooled estimate and to evaluate the robustness of the findings. The result indicated that no single study significantly altered the summary effect size, suggesting the findings are robust. All individual study estimates fell within the 95% confidence interval of the overall polled prevalence, as illustrated in **Figure 5.**

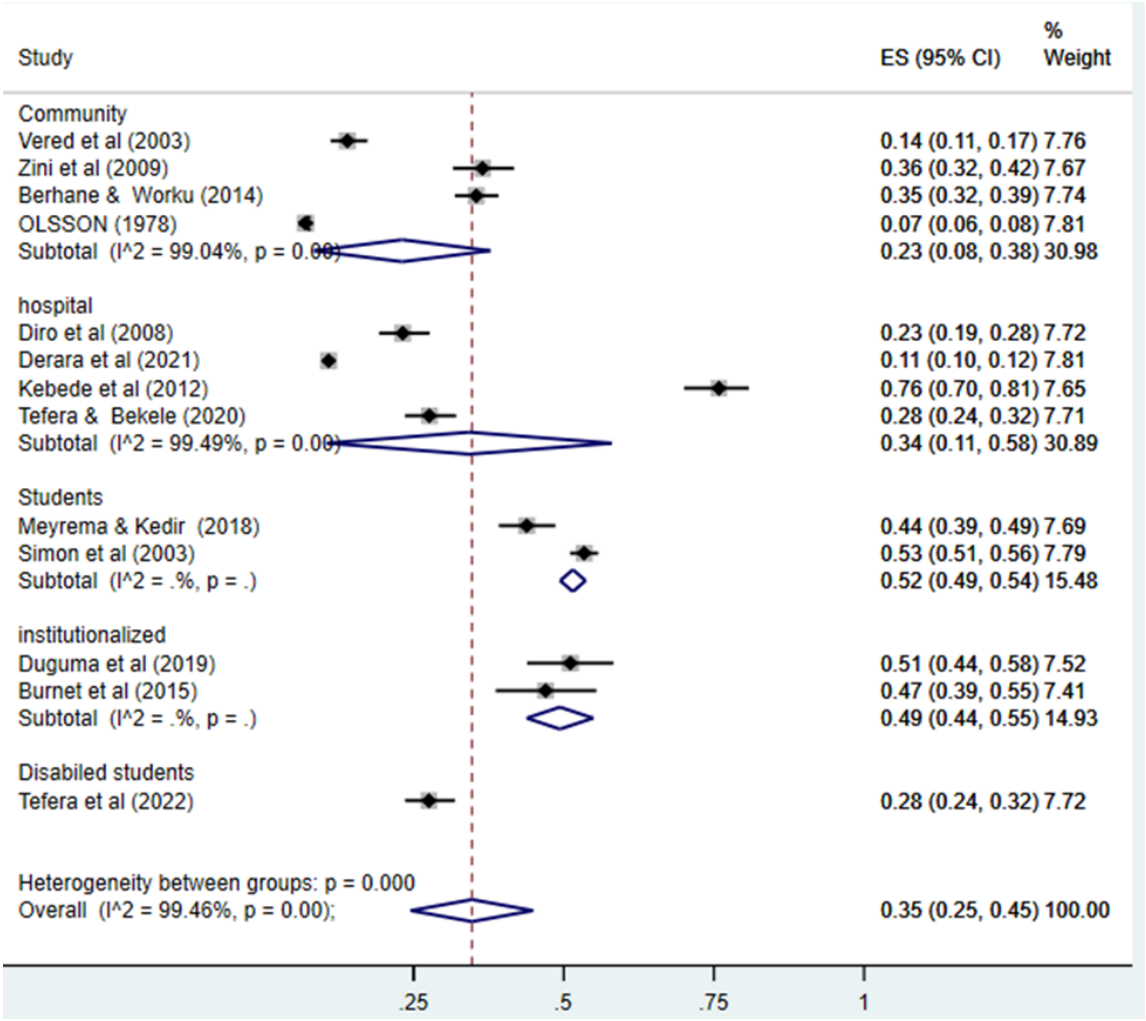

Figure 4: *Subgroup analysis based on the type of study participants.*

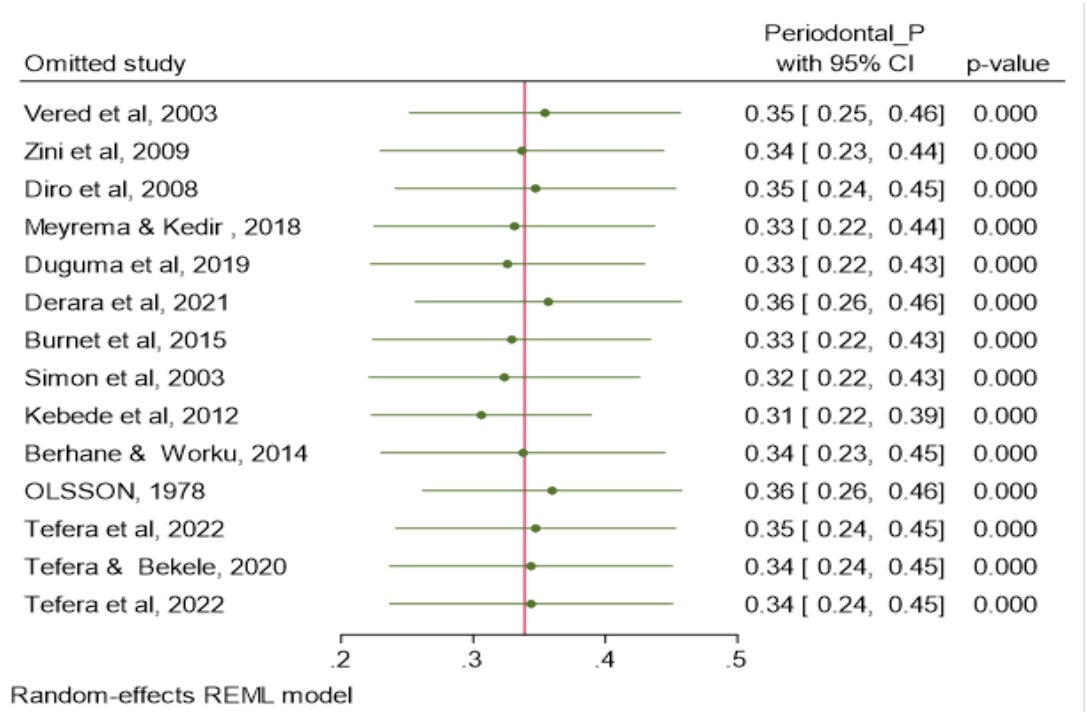

Figure 5: *Sensitivity analysis to show the influential studies of the pooled prevalence (Periodontal_P represents the prevalence of periodontal disease).*

Publication Bias and Trim-and-Fill

The funnel plot showed an asymmetrical distribution of studies, suggesting potential publication bias. This was supported by the result of Egger's regression test, which indicated statistically significant bias (P = 0.0335). In addition, the Doi plot revealed substantial asymmetry, with an LFK index of 5.16, which is more than 2, further confirming the presence of major asymmetry **(Figure 6).**

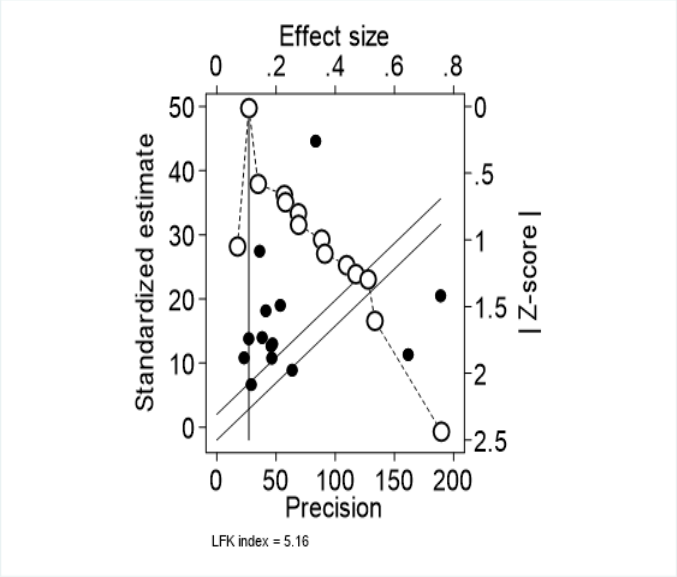

Figure 6: *DOI plot for publication bias assessment*

To address the potential publication bias identified, a trim and fill analysis was performed. This method imputed seven potentially missed studies to adjust for asymmetry in the funnel plot.. After adjusting for publication bias, the revised pooled prevalence of periodontal disease was 13.8% (95%CI: 3.2% to 24.3%), compared to the initial unadjusted estimate of 35%. This substantial difference suggests that the original estimate may have been influenced by publication bias.

Factors associated with Periodontal Disease

A total of eight variables were extracted from the included studies to explore potential predictors of periodontal disease in Ethiopia. Of these, three variables, toothbrush, gender, and carbohydrate intake were found to have a statistically significant association with periodontal disease. Accordingly, individuals who practiced regular toothbrushing had significantly lower odds of developing periodontal disease compared to those who did not brush their teeth (OR = 0.26, 95% CI: 0.24 to 0.28). Similarly, individuals with carbohydrate intake had 46% lower odds of periodontal disease compared to those without carbohydrate intake (OR = 0.54, 95% CI: 0.44 to 0.65). Male participants had 28% lower odds of developing periodontal disease compared to females (OR = 0.72, 95% CI: 0.64 to 0.80) **(Table 3).**

Table 3: Determinants of periodontal disease in Ethiopia

Predictors	Number of articles	AOR	95%CI		I^2	P-value	Q
Toothbrush (reference: No)(26, 28-31)	5	0.26	0.24	0.28	93.3%	0.00	75.12
Smoking (reference: No) (28, 30)	2	1.04	0.65	1.67	0.0	0.439	0.60
Gender (Reference: Female) (26, 27, 29-31)	5	0.72	0.64	0.80	88.7%	0.000	35.28
Carbohydrate intake (Reference: No) (26, 29-31)	4	0.54	0.44	0.65	71.1%	0.008	13.84
Comorbidity (Reference: No) (30, 31)	2	1.94	0.49	7.66	0.0%	0.922	0.01
Dental caries (Reference: No) (30, 31)	2	1.93	0.92	4.07	0.0%	0.781	0.08
Residence (Reference: Rural) (27, 30)	2	0.88	0.72	1.07	91.5%	0.1521	11.73
Income (Reference: >2500ETB) (30, 31)	2	2.16	0.94	4.96	0.0%	0.967	0.00

Risk of bias assessment

The quality of the included studies was evaluated using the Hoy et al tool, and the results were presented in **Table 4.** The risk assessment tool showed that three of the included studies had a moderate risk of bias, and the remaining studies had a low risk of bias.

Table 4: Risk of bias assessment using the Hoy et al. Critical appraisal tool (Yes =1, No=0)

Name of author	External validity				Internal validity						
	Sample representativeness	Representative sampling frame	Random selection or census	Is nonresponse bias minimal?	Data collected directly from subjects?	Acceptable case definition?	Valid and reliable tool for outcome measurement	Is the same mode of data collection for all participants?	Appropriate Length	Were the numerator and denominator appropriate?	Total
Vered et al, 2003(32)	0	1	0	0	1	1	1	1	1	1	7
Zini et al, 2009 (33)	0	1	0	1	1	1	1	1	0	1	7
Diro et al, 2008 (28)	0	0	1	1	1	0	0	1	1	1	6
Meyrema & Kedir, 2018 (34)	1	1	0	1	1	0	0	1	1	1	7
Duguma et al, 2019 (35)	0	1	0	1	1	1	0	1	0	1	6
Derara et al,2021 (27)	0	0	1	1	0	0	0	0	1	1	4
Burnet et al,2-16 (26)	0	0	1	1	1	0	0	1	1	1	6
Simon et al,2003 (29)	1	1	1	1	1	1	1	1	0	1	9
Kebede et al, 2012 (23)	0	1	1	0	1	1	1	1	0	1	7
Berhane & Worku, 2014 (36)	1	0	1	0	1	1	1	1	0	1	7
Olsson, 1978 (22)	1	1	1	1	0	1	1	1	0	1	8
Tefera & Bekele, 2020(30)	1	0	1	1	1	1	1	1	1	1	9
Tefera et al, 2022 (31)	1	0	1	1	1	1	1	1	1	1	9

Discussion

This systematic review and meta-analysis aimed to estimate the pooled prevalence and identify associated factors of periodontal disease in Ethiopia. The findings revealed that approximately **35%** (95% CI: 25%–45%) of the Ethiopian population is affected by periodontal disease, highlighting a substantial public health concern. Notably, a high level of heterogeneity was observed across the included studies (**I^2 = 99.46%**). Key predictors identified were gender, **carbohydrate intake,** and tooth brushing practice.

The prevalence found in this study is lower than reported in India,51% (5) and the United States (47%) (6). One possible explanation for this difference is the age profile of participants: over three-fourths of the Ethiopian studies involved children and young adults, while the Indian and U.S studies primarily focused on adults and elderly populations, in whom periodontal disease. As the global population ages and tooth retention increases, the burden of periodontitis is expected to rise, especially in settings where geriatric oral health services are lacking (4, 21, 37).

The current study revealed that males were less likely to develop periodontal disease compared to females. This result is inconsistent with studies done in diabetic populations, such as one that reported higher odds among males (38), and another study from Japan (39). The discrepancy may be attributed to differences in study populations, particularly the presence of systemic conditions such as diabetes mellitus, which is a well -established risk factor for periodontal disease and may modify the gender-related risk. Furthermore, in Ethiopian communities, men may use traditional oral hygiene methods like chewing sticks (locally known as Mefakia) more consistently than women (40, 41).

Regarding oral hygiene practice, our meta-analysis showed that regular tooth brushing significantly reduced the odds of periodontal disease (OR = 0.26, 95% CI: 0.24 to 0.28). This aligns with findings by Lertpimonchai et al. (42), who reported that brushing twice daily reduced the risk of periodontitis by 34%. Similarly, Zimmermann et al. found that individuals with infrequent tooth brushing practice had higher odds of periodontal disease (43). These consistent results across studies may be explained by the role of toothbrushing in preventing plaque and calculus accumulation, which are key etiological factors in the pathogenesis of periodontal disease (44).

Subgroup analysis revealed regional and population-based differences in the prevalence of periodontal disease. The highest prevalence was reported in Addis Ababa, with a pooled estimate of 42% (95%CI: 29% to 55%). This elevated prevalence may be influenced by the characteristics of the study populations in the region, as many participants were institutionalized and older adults. Similarly, institutionalized participants and students had a higher prevalence compared to community-based studies. A comparable result was reported in Italy, where prolonged institutionalization was associated with poorer oral hygiene and a higher burden of untreated periodontitis, highlighting the vulnerability of this population to periodontal conditions(45). This may be attributed to limited access to dental care, lower oral health awareness, and reduced autonomy in maintaining daily oral hygiene practices within institutional settings(46).

In interpreting the findings of this review, it is important to consider the potential influence of publication bias and study heterogeneity. Evidence of publication bias was indicated by the asymmetry in the funnel plot and confirmed by Egger's test (p = 0.0335) and the DOI plot (LFK index = 5.16), suggesting a major risk of bias. The trim-and-fill analysis imputed seven missing studies and adjusted the pooled prevalence downward to 13.8% (95% CI: 3.2%–24.3%), a substantial reduction from the original estimate. The substantial reduction in pooled prevalence after applying the trim-and-fill method suggests that the initial estimate may have been inflated due to **publication bias**, where studies reporting higher prevalence are more likely to be published.

In addition to publication bias, considerable heterogeneity was observed among the included studies (I^2 = 99.46%). This high level of inconsistency likely reflects differences in study populations, settings (e.g., community-based vs. institutionalized), and methodological approaches. Interestingly, subgroup analysis revealed minimal heterogeneity among studies conducted in institutionalized populations (I^2 = 0.02%), suggesting that participant type may be a major source of variability. The relatively uniform conditions within institutional settings may lead to more consistent findings, while community-based studies may capture a broader and more diverse range of exposures and outcomes. Given these sources of bias and variability, the overall pooled prevalence should be inter-

preted with caution. Future primary studies employing standardized methodologies and more representative samples are needed to produce more robust and generalizable estimates of periodontal disease burden in Ethiopia.

Strengths and limitations

This review has several notable strengths. First, it represents the first national-level review to synthesize available evidence on the prevalence and predictors of periodontal disease in Ethiopia, addressing a critical gap in oral health research in the country. Second, a standardized risk of bias assessment tool was employed to evaluate the quality of the included studies, which enhances the reliability of the synthesized findings.

Despite its strengths, this review has some limitations that should be acknowledged. First, there was an underrepresentation of certain regions in Ethiopia, which may limit the generalizability of the findings to the national population. Second, there was variation in the diagnostic criteria and assessment tools used across studies, which could have introduced inconsistencies in case definition and measurement of periodontal disease. Third, the lack of a national oral health surveillance system limits the ability to draw firm conclusions about the true burden of periodontal disease across the country. Fourth, data collection was conducted by both dental professionals and other healthcare workers, potentially resulting in variability in examiner reliability and diagnostic accuracy. Finally, the high level of heterogeneity observed in the meta-analysis further underscores the need for cautious interpretation and highlights the importance of standardized methodologies in future research.

Conclusion and recommendation

This systematic review and meta-analysis revealed that approximately one in three Ethiopians is affected by periodontal disease, with a pooled prevalence of 35%. The burden is particularly higher among institutionalized populations and in Addis Ababa. Key predictors of periodontal disease included poor toothbrushing habits, female gender, and lack of carbohydrate intake. The consistent association between oral hygiene practices and disease risk underscores the urgent need for targeted preventive strategies. The high heterogeneity and evidence of publication bias suggest variability in local practices and diagnostic methods, highlighting the need for standardized national guidelines and surveillance systems. Public health efforts should focus on improving oral health awareness, promoting proper oral hygiene practices such as regular tooth brushing, and encouraging healthy dietary habits.

Declarations

Author Contributions

Conceptualization: Amare Teshome, Nebiyou Bekele, Martha Solomon, Shegaye Shumet, Tigist Mulugeta

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Writing – review and editing: Amare Teshome, Nebiyou Bekele, Martha Solomon, Shegaye Shumet, Tigist Mulugeta, Dessie Abebaw. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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Availability of Data and Materials: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request (teferaden@gmail.com).

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