JOURNAL OF NATURAL AND APPLIED SCIENCES RESEARCH

ISSN (In process)

IUO-JOURNAL OF NATURAL AND APPLIED SCIENCES RESEACH (JNASR)

VOL 1, ISSUE 1
May, 2025

Published by the College of Natural and Applied Sciences,
Igbinedion University, Okada, NIGERIA
jnasr@iuokada.edu.ng

BASIC JOURNAL INFORMATION

MAIN TITLE:	IUO-Journal of Natural and Applied Sciences Research (JNASR)
Abbreviated TITLE:	JNASR
ISSN:	(in process)
URL:	https://www.jnasr.iuokada.edu.ng
COUNTRY:	Nigeria
FREQUENCY:	Bi-annual
AVAILABILITY:	Open source, international
Editor-in-Chief:	Rev. Professor Longy O. Anyanwu, Ed.D., FCPN, FCAARS, FCSP(USA)
Publisher:	College of Natural and Applied Sciences, Igbinedion University, Okada, Edo State, Nigeria

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welcomes the submission of solicited and/or unsolicited manuscripts. We welcome submissions that focus on any aspect of natural and/or applied sciences. We also welcome some book reviews, case comments and public lectures. Selected articles would be published online and in hard copy and also prominently displayed on the institutional website as a resource for scholars and practitioners worldwide. Papers must be based on original research, conformant to the usual standard of academic publishing, fully referenced and presented in the standard format employed by the JNASR. Papers will be evaluated based on their contemporary relevance, contribution to their selected fields of research, structure and analytical vigor.

Journal of Natural and Applied Sciences Research (JNASR)
Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Published by the College of Natural and Applied Sciences, Igbinedion University, Okada, Nigeria.

First printing/publishing, 2025.
URL: https://www.jnasr.iuokada.edu.ng

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Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Editorial Preface

Welcome to the maiden issue (Vol. 1, Issue 1) of the IUO-Journal of Natural and Applied Sciences Research (JNASR), May 2025. This issue presents a diverse and comprehensive collection of research that spans a range of vital topics and issues within the sciences and technology. As we explore into the uncharted span of knowledge, this maiden issue features insightful articles contributed by scholars from a variety of fields in the sciences and technology. The contributions provide fresh perspectives on contemporary issues, offering valuable knowledge for academics, policymakers, and practitioners alike. The authors offer a comprehensive analysis of the societal challenges and opportunities, such as "Electroosmotic and Thermal Analysis of Magnetohydrodynamic Couple-Stress Hybrid Nanofluid Flow in a Porous Medium with Hall and Ion-Slip Effects", "Antimicrobial Resistance in Pregnancy: Prevalence and Characterization of ESBL-Producing Uropathogens in a Nigerian Tertiary Hospital", and "An Enhanced Framework for Directing and Mitigating Ransomware Attacks in IoT/IoMT-Based Healthcare Networks" to "Prevalence and Aetiological Spectrum of Transaminitis among Patients with Non-Viral Hepatitis in a Rural Tertiary Hospital in Southern Nigeria".

Another article in this issue addresses key global concerns, such as "Biomass Waste Valorization: Exploring New Fronteirs as Biochemists." Yet other investigations richly encompass "Exploring Multidrug-Resistant Staphylococci on Community Surfaces: A MALDI-TOF MS Analysis from Nigeria", "Enzymatic Biomass Transformation; Revolutionizing Renewable Energy", and "Evaluating Police Awareness and Utilization of Forensic Science in Criminal Investigations: Evidence from the Edo State Police Command, Nigeria". These articles provide significant insights into the factors that contribute to successful prosecution of crimes. In addition, we feature articles that examine the "Mechanical, Electronic, Vibrational, Structural, and Thermodynamic Properties of ZrNiX (X-Pb, Sn) Half-Heusler alloys", "Prevalence and Risk Factors of High-Risk HPV E6 /E7 Oncoproteins Among Women in Benin-City, Nigeria: Implications for Cervical Cancer Prevention", and "Detection of Klebsiella variicola, Escherichia coli and Providentia staurti in satchet water sold in Okada metropolis, Ovia North LGA,Edo state Nigeria".

As always, JNASR is committed to promoting high-quality, interdisciplinary research that addresses both current and future challenges in the global society as evidenced by virtually all the articles in this publication. This issue exemplifies our ongoing dedication to contributing to the global knowledge base and to fostering collaboration across countries and disciplines. We believe that all the papers published in this special issue will have great influence on knowledge search and practice in the sciences and technology. Approximately 70% of article submissions have been rigorously reviewed and accepted for publication in this this special issue.

We are grateful to all of our contributors for their rigorous research and thoughtful analysis, and we hope that the ideas presented in this issue will spark further discussion, innovation, invention, and policy development in the sciences, technology, and other areas. Thank you for your continued support of JNASR. We look forward to bringing you more impactful research in the coming issues.
Enjoy the reading.

Rev. Professor Longy O. Anyanwu, Ed.D, , FCPN, FCSP(USA), FCAARS
Editor-in-Chief
IUO_Journal of Natural and Applied Sciences Research (JNASR)
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a) **Volume:** 01**, Issue:** 02
b) **Last Date:** 30th October, 2025
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(JNASR) Journal of Natural and Applied Sciences Research
Ibezim *et al.*, (2025). 1(1): 1-8. Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Prevalence and Aetiological Spectrum of Transaminitis among Patients with Non-Viral Hepatitis in a Rural Tertiary Hospital in Southern Nigeria

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Abstract:
Non-viral hepatitis remains a significant health concern in rural populations, where exposure to hepatotoxic substances is common, and healthcare resources are often limited. This study examined the aetiological distribution and associated transaminase patterns among 100 patients diagnosed with non-viral hepatitis in a rural Nigerian setting. Diagnoses were grouped into seven clinical categories: alcoholic hepatitis (20%), herbal toxicity (20%), drug-induced liver injury (15%), aflatoxin exposure (15%), autoimmune hepatitis (10%), parasitic infection (10%), and protein deficiency (10%). Alanine aminotransferase (ALT) levels were more frequently reduced than elevated. Protein deficiency (20%) and DILI (17%) were the leading causes of reduced ALT, whereas elevated ALT was more associated with DILI (10%) and autoimmune hepatitis (4%). A significant inverse correlation was observed between aetiology and ALT levels ($\tau_\beta = -0.337$, $p = 0.003$). Aspartate aminotransferase (AST) was predominantly elevated across most aetiologies. The highest elevations were seen in alcoholic hepatitis (20%), DILI (20%), herbal toxicity (15%), and aflatoxin exposure (15%). A stronger inverse correlation was found between AST levels and aetiology ($\tau_\beta = -0.477$, $p < 0.001$), suggesting AST may be more sensitive to certain toxic and inflammatory liver insults. These findings show the diagnostic utility of transaminase patterns in non-viral liver disease and reveal the contribution of preventable causes in rural settings. However, clinicians must apply a multidimensional approach to liver function interpretation by integrating biochemical data with clinical signs to ensure accurate diagnosis and optimal patient care with public health interventions aimed at controlling alcohol use, unregulated herbal remedies, and unsafe drugs.

Keywords: Transaminitis, liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alcoholic Hepatitis, Herbal Toxicity, Drug-Induced Liver Injury (DILI)

Introduction:

Transaminitis refers to an abnormal elevation in serum liver transaminase enzymes, primarily alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are sensitive indicators of hepatocellular injury or inflammation. These enzymes are predominantly found in hepatocytes, and their leakage into the bloodstream typically reflects liver cell damage, although they may also be elevated in other systemic conditions (Navarro & Senior, 2006). Traditionally, the most widely recognised causes of transaminitis are viral hepatitides, specifically hepatitis A, B, and C, which continue to pose a significant public health burden worldwide, especially in low- and middle-income countries.

However, a growing body of evidence suggests that non-viral causes are increasingly contributing to liver enzyme abnormalities, particularly in developing nations such as Nigeria. Non-viral aetiologies of transaminitis encompass a broad spectrum of conditions, including non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, drug-induced liver injury (DILI), autoimmune hepatitis, aflatoxin exposure, protein-energy malnutrition, and hepatotoxicity from unregulated herbal remedies (Chalasani *et al.*, 2012; Okenwa *et al.*, 2023; Ofori-Asenso & Agyeman, 2016). NAFLD, for instance, is emerging as a major cause of

liver disease globally and is now recognised as the hepatic manifestation of metabolic syndrome, with increasing prevalence among individuals with obesity, diabetes, and dyslipidaemia (Nwokediuko, 2005; Ogbera &Azenabor, 2010). Similarly, herbal and traditional remedies, widely consumed in sub-Saharan Africa due to cultural beliefs and limited access to formal healthcare, are often hepatotoxic due to unregulated formulations and undisclosed ingredients. In many clinical settings across sub-Saharan Africa, including Nigeria, diagnostic protocols are often skewed towards identifying viral hepatitis, while non-viral causes of liver dysfunction remain under-recognised. This imbalance may delay accurate diagnosis, appropriate intervention, and epidemiological understanding of liver disease burdens in these regions. Moreover, many patients with elevated liver enzymes are treated empirically or inadequately investigated due to limited diagnostic infrastructure, inconsistent record-keeping, and poor follow-up systems in public healthcare facilities. Despite the clinical importance of identifying the underlying cause of transaminitis, especially in patients who test negative for common hepatotropic viruses, there remains a dearth of local data addressing the prevalence and aetiological spectrum of non-viral transaminitis in tertiary care centres. Existing studies have primarily focused on viral hepatitis seroprevalence, with minimal emphasis on biochemical and clinical patterns of non-viral liver injury (Paruk *et al*., 2019).

This study, therefore, aims to evaluate the prevalence, demographic distribution, and aetiological profile of transaminitis in patients who are seronegative for hepatitis A, B, and C, as seen at a tertiary hospital in southern Nigeria. By shedding light on the non-viral drivers of liver dysfunction in this setting, the findings are expected to enhance clinical awareness, promote early detection, and guide appropriate management strategies tailored to the local disease burden.

Methods:

Study Design

This retrospective cross-sectional study was conducted at the Department of Internal Medicine, Igbinedion University Teaching Hospital, in collaboration with the Department of Biochemistry, Igbinedion University, Okada, Nigeria. The study covered medical records from January 2023 to March 2025. Medical records of 100 adult patients who presented with abnormal liver function tests (LFTs) and tested negative for hepatitis A, B, and C were reviewed.

Inclusion criteria:

- Age ≥18 years
- Abnormal alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) levels
- Seronegative for hepatitis A, B, and C

Exclusion criteria:

- Age <18 years
- Positive viral hepatitis serology
- Incomplete LFT records

Data Collection

Demographic data, clinical presentation, risk factors (such as alcohol intake and drug history), and final diagnosis were extracted from hospital records using a standardised data abstraction form. For analytical purposes, final diagnoses were categorised into clinically relevant aetiological groups, including alcoholic hepatitis, drug-induced liver injury (DILI), autoimmune hepatitis, aflatoxin exposure, parasitic infection, protein deficiency, and herbal toxicity. This classification was done jointly by the Medical Officer and reviewed by a Chief Consultant in collaboration with the Department of Biochemistry to ensure consistency in diagnostic grouping.

Biochemical Analysis

Serum ALT and AST levels were determined in the hospital's laboratory using an automated Roche Cobas® c111 analyser, following the International Federation of Clinical Chemistry (IFCC) standardised enzymatic colorimetric method, which measures enzyme activity at 37°C with optimised substrate concentrations for precision.

The normal reference ranges used were based on IFCC recommendations (Schumann *et al*., 2002):

- ALT (Alanine aminotransferase): 7–56 U/L
- AST (Aspartate aminotransferase): 5–40 U/L

These values were consistent with those adopted by the World Health Organization (WHO) and IFCC collaborative protocols, as well as those referenced in the instrument manufacturer's manual (Roche Diagnostics, 2009; World health Organization, 2017).

Transaminitis was defined as ALT and/or AST elevation ≥1.5 times the upper limit of normal (ULN), in line with commonly accepted clinical thresholds for hepatocellular injury severity classification.

Statistical Analysis

Data were analysed using IBM SPSS Statistics version 26. Descriptive statistics were used to summarise the data, and results were expressed as means, frequencies, and percentages.

This sample size of 100 was deemed sufficient based on similar published retrospective studies in Nigeria investigating transaminitis and non-viral hepatic causes (Adekanle *et al.*, 2020).

Ethical Approval

Ethical approval for this study was obtained from the Health Research Ethics Committee (HREC) of Igbinedion University Teaching Hospital (IUTH), Okada, Edo State, Nigeria, with protocol number IUTH/R.24/VOL.I/156. Patient confidentiality was maintained by anonymising all data, and the study adhered to the ethical principles outlined in the Declaration of Helsinki for research involving human subjects (World Medical Association, 2013).

RESULTS

Aetiologies of Liver Injury in Non-Viral Hepatitis Patients

Out of the 100 patients included in the study, the most common identified causes of non-viral hepatitis were alcoholic hepatitis and herbal toxicity, each accounting for 20% of cases. This was followed by aflatoxin exposure and drug-induced liver injury (DILI), which each constituted 15% of the study population. Autoimmune hepatitis, parasitic infections, and protein deficiency were the least frequent causes, each contributing 10% to the overall distribution. This distribution highlights the significant burden of modifiable or preventable aetiologies, such as alcohol use and herbal medication use in non-viral hepatic dysfunction within the study setting.

Table 1: Aetiologies of Liver Injury in Non-Viral Hepatitis Patients

AETIOLOGY	FREQUENCY (N)	PERCENT (%)
Aflatoxin Exposure	15.0	15.0
Alcoholic Hepatitis	20.0	20.0
Autoimmune Hepatitis	10.0	10.0
Drug-Induced Liver Injury	15.0	15.0
Herbal Toxicity	20.0	20.0
Parasitic Infection	10.0	10.0
Protein Deficiency	10.0	10.0
Total	100.0	100.0

Relationship between ALT and Aetiologies of Liver Injury in Non-Viral Hepatitis Patients

The clustered bar chart in Figure 1 illustrates the distribution of different aetiologies of liver injury in relation to ALT (Alanine Aminotransferase) levels, categorised as either elevated or reduced, among 100 patients. A significant association was observed between aetiology and ALT levels ($\chi^2 = 56.574$, $p < 0.001$), with a negative correlation noted (Kendall's $\tau = -0.337$, $p = 0.003$).

Overall, reduced ALT levels were more prevalent than elevated levels across nearly all aetiological categories. The most prominent finding is that protein deficiency accounted for the highest number of cases with reduced ALT, representing 20 patients (20%). This was followed by drug-induced liver injury (17 patients; 17%) and herbal toxicity (15 patients; 15%) in the reduced ALT group.

In contrast, elevated ALT levels were most commonly observed in cases of drug-induced liver injury (10 patients; 10%) and autoimmune hepatitis (4 patients; 4%). Other aetiologies such as aflatoxin exposure, alcoholic hepatitis, and parasitic infection each accounted for 3%–4% of the elevated ALT cases.

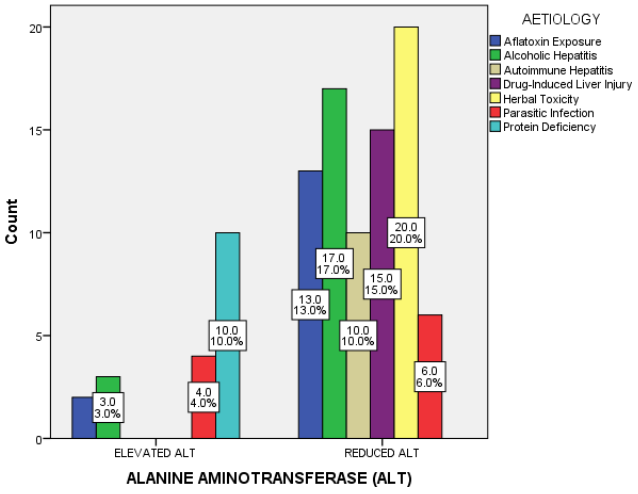

Figure 1: Relationship between ALT and Aetiologies of Liver Injury in Non-Viral Hepatitis Patients (elevated ALT >56U/L, reduced ALT <7U/L)

Relationship between AST and Aetiologies of Liver Injury in Non-Viral Hepatitis Patients

The clustered bar chart in Figure 2 illustrates the distribution of elevated and reduced Aspartate Aminotransferase (AST) levels across various aetiologies among 100 patients. A significant association was observed between aetiology and AST levels ($\chi^2 =$

61.000, $p < 0.001$). Also, a statistically significant negative correlation was found between AST levels and aetiology (Kendall's tau-b = –0.477, $p < 0.001$), suggesting that specific aetiologies are associated with a higher likelihood of AST elevation.

A majority of the cases showed elevated AST levels, particularly in patients diagnosed with alcoholic hepatitis (20%), drug-induced liver injury (DILI) (20%), herbal toxicity (15%), aflatoxin exposure (15%), protein deficiency (9%), autoimmune hepatitis (10%). In contrast, reduced AST levels were far less common and were mostly observed in: protein deficiency (10%) and parasitic infection (1%)

This pattern suggests that AST elevation is more prominent in hepatocellular injury from toxic, infectious, or inflammatory causes, with alcoholic hepatitis, drug-induced liver injury, and herbal toxicity being the leading contributors in this sample. The predominance of elevated AST reinforces its role as a marker of liver injury in non-viral hepatic conditions.

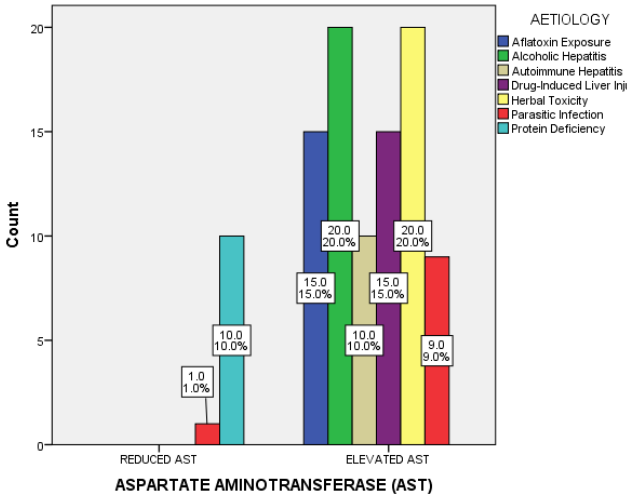

Figure 2: Relationship between AST and Aetiologies of Liver Injury in Non-Viral Hepatitis Patients

(elevated AST >40U/L, reduced AST <5U/L)

DISCUSSION

Aetiologies of Liver Injury in Non-Viral Hepatitis Patients

The observed distribution of non-viral hepatitis aetiologies in this study was seen as alcoholic hepatitis and herbal toxicity, each accounting for 20% of cases, followed by aflatoxin exposure and drug-induced liver injury (DILI) at 15% each, and autoimmune hepatitis, parasitic infections, and protein deficiency at 10% each.

The significant proportion of alcoholic hepatitis among patients in rural settings may be attributed to several factors, such as cultural practices and socioeconomic factors. In many rural communities, alcohol consumption is deeply ingrained in social and cultural practices. Locally brewed alcoholic beverages are often more accessible and affordable than regulated commercial products, leading to higher consumption rates. There is often limited awareness about the hepatotoxic effects of excessive alcohol intake. This lack of knowledge contributes to continued consumption despite the associated health risks. Also, rural areas frequently have inadequate healthcare infrastructure, making it challenging for individuals to receive education on the dangers of alcohol abuse and to access treatment for alcohol-related disorders. This is entirely devastating, as a study by Ndububa *et al.* highlighted alcohol consumption as an independent determinant in the progression of chronic liver disease in Nigeria, emphasising the role of alcohol in hepatic pathology within the region (Ndububa *et al*., 2005). The equal prevalence of herbal toxicity reflects the widespread use of traditional herbal medicines in rural areas (Auerbach *et al*., 2012). Due to limited access to conventional healthcare and poor health awareness deep-rooted in strong cultural beliefs and myths, rural populations often depend on traditional healers and herbal remedies for the treatment of various ailments. Herbal medicines are commonly perceived as natural and safe, leading to their unregulated use without consideration of potential hepatotoxic effects. Many herbal products are prepared without standardised dosages or quality control, increasing the risk of contamination with hepatotoxic substances. Research indicates that herbal medicine use is associated with significant liver fibrosis in both HIV-infected and uninfected individuals in sub-Saharan Africa. Additionally, the National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria has reported that high toxic content in herbal medicines can damage vital organs, including the liver (Auerbach *et al*., 2012; Punch Newspapers, 2020).

The notable incidence of aflatoxin-related hepatitis is likely due to the consumption of staple foods such as maize and groundnuts, which are susceptible to aflatoxin contamination, in rural areas. Inadequate storage conditions facilitate the growth of aflatoxin-producing fungi, increasing exposure risk. Furthermore, limited knowledge about aflatoxin contamination and insufficient food safety regulations contribute to ongoing exposure. Studies have shown that aflatoxin exposure is a significant risk factor for liver disease in regions where contaminated food storage is prevalent (Amadi & Orisakwe, 2018). The occurrence of DILI in rural

populations may stem from self-medication practices, which may be due to limited access to healthcare professionals; individuals often self-medicate with over-the-counter drugs, increasing the risk of hepatotoxicity. Likewise, the availability of counterfeit or substandard medications in rural markets can lead to liver injury, as well as inadequate monitoring of drug safety and adverse effects, which also contributes to the prevalence of DILI (Amadi & Orisakwe, 2018). A case series from a single centre reported that herb-induced liver injury, though less documented, is a growing problem, highlighting the need for careful assessment of herbal medicine use (Dağ *et al*., 2014).

Underdiagnosis of autoimmune hepatitis is common in rural areas due to limited access to specialised diagnostic tools and healthcare professionals, which may have been the reason for the low output in our findings. Also, while parasitic infections are prevalent, their progression to liver disease may be less frequent or underreported. Malnutrition, including protein deficiency, is prevalent in rural areas due to food insecurity, but its direct link to liver disease may not be well recognised or documented. Also, patients with protein-losing diseases like nephrotic syndrome or glomerulopathies in general might have the same presentation. The underreporting and lack of documentation of these conditions in rural settings contribute to their perceived lower prevalence (Amadi & Orisakwe, 2018).

Relationship between ALT and Aetiologies of Liver Injury in Non-Viral Hepatitis Patients

Transaminitis refers to elevated levels of liver enzymes, specifically ALT and aspartate aminotransferase (AST), indicating hepatocellular injury. ALT is primarily localised in the liver, making it a more specific marker for liver damage compared to AST, which is also found in other tissues such as the heart and muscles. Elevations in these enzymes typically suggest active liver inflammation or injury. However, the prevalence of reduced ALT levels in this study suggests alternative or additional pathophysiological mechanisms at play.

Severe protein malnutrition can lead to diminished synthesis of liver enzymes, including ALT. In protein-deficient states, the liver's capacity to produce these enzymes is compromised, leading to lower serum levels. This phenomenon has been observed in malnourished populations, where liver enzyme levels may not accurately reflect the extent of hepatic injury (Abulude *et al*., 2017). While DILI often presents with elevated liver enzymes, certain hepatotoxic drugs can cause mitochondrial dysfunction and hepatocellular necrosis without significant enzyme release, especially in chronic exposure scenarios. Additionally, some drugs may impair protein synthesis, leading to reduced enzyme production. The European Association for the Study of the Liver (EASL) notes that serum aminotransferase levels remain the mainstay for detecting and classifying liver damage in suspected DILI, but variations can occur depending on the drug and individual response (European Association for the Study of the Liver, 2019). Also, the use of hepatotoxic herbal remedies, common in certain regions, can lead to liver injury with variable enzyme patterns. Some herbal toxins may cause liver damage without significant enzyme elevation, particularly if the injury leads to impaired enzyme synthesis. This shows the importance of considering herbal medicine use in patients with liver dysfunction, even when standard liver enzymes are not elevated (Dağ *et al*., 2014). Furthermore, autoimmune hepatitis typically manifests with elevated transaminases due to ongoing immune-mediated hepatocellular inflammation. Persistent elevation of ALT is a hallmark of active disease and guides treatment decisions.

The predominance of reduced ALT levels in this study highlights the need for a careful interpretation of liver enzyme results. Relying solely on elevated transaminases to detect liver injury may lead to underdiagnosis, especially in conditions like protein deficiency or certain types of DILI and herbal toxicity. Comprehensive clinical assessment, including nutritional evaluation and detailed medication and herbal remedy histories, is essential for accurate diagnosis.

Relationship between AST and Aetiologies of Liver Injury Injury in Non-Viral Hepatitis Patients

In alcoholic hepatitis, AST levels often exceed ALT levels, typically with an AST/ALT ratio greater than 2:1. This pattern results from alcohol-induced mitochondrial injury and a relative deficiency of pyridoxal phosphate (vitamin B6), which affects ALT synthesis more than AST (Botros & Sikaris, 2013). Also, DILI can present with varying patterns of enzyme elevation, depending on the offending agent. Some drugs cause a hepatocellular injury pattern with predominant AST elevation. For instance, acetaminophen toxicity often leads to significant increases in AST levels, which is predominantly abused in rural communities (Francis & Navarro, 2024). In the same way, herbal-induced liver injury (HILI) is a growing concern, especially in regions with prevalent use of traditional medicines. Certain herbal compounds can cause hepatocellular damage, leading to elevated AST levels. The pattern of enzyme elevation in HILI varies, but significant AST increases

have been documented (Nunes *et al*., 2022). Furthermore, aflatoxins, produced by certain fungi, are potent hepatotoxins. Exposure can lead to acute liver injury characterised by elevated AST levels. Studies have shown that aflatoxin B1 exposure results in significant increases in AST, reflecting hepatocellular necrosis (Dhakal *et al*., 2023). Severe protein malnutrition can also impair liver function, leading to altered enzyme synthesis and release. While data are limited, some studies suggest that protein deficiency may result in elevated AST levels due to compromised hepatocellular integrity. Autoimmune hepatitis is characterised by immune-mediated hepatocellular inflammation, leading to elevated transaminases. Both AST and ALT levels are typically increased, but AST may be more prominently elevated in some cases (Linzay *et al*., 2023).

The predominance of elevated AST (aspartate aminotransferase) levels observed across various aetiologies in this study exposes the critical role of AST in the biochemical assessment of hepatic dysfunction, particularly in non-viral liver diseases. While both AST and ALT (alanine aminotransferase) are markers of hepatocellular injury, AST is less specific to the liver due to its presence in other tissues such as skeletal muscle, cardiac muscle, kidneys, and the brain. This broader tissue distribution means that elevated AST levels can arise from both hepatic and extrahepatic sources, including muscle trauma, myocardial infarction, and hemolysis (Botros & Sikaris, 2013). Therefore, clinicians are advised to interpret AST and ALT elevations alongside patient history, physical examination findings, imaging studies, and additional liver function parameters, such as alkaline phosphatase, bilirubin, albumin, and prothrombin time. For instance, in herbal toxicity or drug-induced liver injury (DILI), elevated AST might reflect active hepatocellular necrosis, but further confirmation via imaging (e.g., ultrasound or CT scan) and possibly liver biopsy may be required for definitive diagnosis and management.

Conclusion

This study unveils the biochemical and aetiological profiles of non-viral hepatitis among a rural population, with a particular focus on transaminase patterns. The findings reveal alcoholic hepatitis and herbal toxicity as the most common causes of liver dysfunction in this setting. This highlights the continued burden of modifiable and preventable risk factors in rural communities, where unregulated herbal medicine use, alcohol abuse, and nutritional deficiencies remain prevalent. Notably, elevated AST levels were more commonly observed than ALT across multiple aetiological categories. This suggests a predominance of hepatocellular injury, potentially complicated by systemic factors such as malnutrition, coexisting muscle injury, or chronic alcohol intake. The disproportionate elevation of AST over ALT, particularly in alcoholic hepatitis and drug-induced liver injury, exposes the relevance of AST as an important, though less liver-specific, marker of hepatic injury. Conversely, reduced transaminase levels, especially ALT, were seen in conditions like protein deficiency, possibly reflecting diminished hepatocyte synthetic function.

The study reinforces the importance of contextual interpretation of liver enzymes. AST and ALT levels should not be assessed in isolation but must be integrated with clinical, nutritional, and epidemiological data, especially in resource-limited environments.

Recommendations and Public Health Policies

1. **Community Education & Public Health Campaigns**: There is an urgent need for targeted health education programs in rural areas to raise awareness about the dangers of excessive alcohol use and unsupervised herbal medicine consumption.
2. **Regulation of Herbal Products**: Regulatory authorities should implement stricter controls on the production, sale, and advertisement of herbal remedies, with routine screening for hepatotoxic compounds.
3. **Routine Liver Function Screening**: In at-risk populations, particularly those with known exposure to hepatotoxins (e.g., alcohol, traditional herbs, aflatoxins), periodic liver enzyme screening should be promoted to allow for early detection of liver damage.
4. **Nutritional Interventions**: Since protein deficiency featured prominently in patients with reduced transaminase levels, community-level nutritional support and supplementation programs are warranted.
5. **Further Research**: Additional studies involving larger, multicentric rural populations with more extensive biochemical and histological assessments are recommended to validate and expand on these findings.
6. **Capacity Building for Rural Clinicians**: Training primary healthcare workers on the interpretation of transaminase levels, particularly in the context of rural liver disease, will enhance diagnostic accuracy and patient management.

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(JNASR) Journal of Natural and Applied Sciences Research
Okwu *et al.*, (2025). 1(1): 9-24. Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Exploring Multidrug-Resistant Staphylococci on Community Surfaces: A MALDI-TOF MS Analysis from Nigeria

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Abstract

Recently, there is an apprehension of the increasing likelihood of contaminated fomites to mediate the transmission of infectious diseases, especially caused by staphylococci, to and from humans. The present study sought to estimate the prevalence of staphylococci and their multidrug-resistant species on fomites collected from Okada town situated in Edo State, Nigeria. A total of 250 swab samples were randomly collected from fomites (tables, chairs, door handles, keys, equipment, switches and handrails). Isolation of staphylococcal colonies was done by streak plate technique. Presumptive staphylococci colonies were initially identified by standard phenotypic tests, with the suspected staphylococcal isolates subsequently confirmed by Matrix-assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry. Confirmed isolates were tested for multidrug resistance by Kirby Bauer disc diffusion test. The overall estimated prevalence of fomites contaminated with *Staphylococcus* species was estimated at 15.60% (39/250), with door handles accounting for the most contaminated fomites. Speciation by MALDI-TOF MS identified *S. haemolyticus, S. kloosii*, *S. nepalensis*, *S. saprophyticus*, *S. sciuri*, *S. simulans* and *S. xylosus*, with *S. sciuri* occurring most frequently on the fomites. All staphylococcal colonies were resistant to all beta-lactam antibiotics tested, but were generally most sensitive to fluoroquinolone (ciprofloxacin) and aminoglycoside (gentamicin) classes of antibiotics. The likelihood of contamination of fomites with multidrug-resistant staphylococci was estimated at 11.20% (28/250). The contaminated fomites were found to be a potential source of multidrug-resistant staphylococci since the reported MAR (0.79) exceeded the recommended limit (0.2). Appropriate hygienic measures to mitigate potential staphylococcal cross-contamination mediated by the contaminated fomites are recommended to avoid potential significant health risks in the future.

KEYWORDS: Antibiotics, Fomite, MALDI-TOF MS, Multidrug-resistant staphylococci, Multiple antibiotic resistance index (MAR).

Introduction

Fomites refer to inanimate objects that are capable of spreading infections upon contamination with pathogenic microbes (Barrie *et al*., 1994; Nwankwo, 2012). Several epidemiological studies have revealed that fomites are important vehicles for transmission of human pathogens in environments such as sports facilities, child-care facilities, hospitals, as well as other outdoor and indoor environments (Bloomfield & Scott, 1997; Bures *et al*., 2000; Al-Harbi *et al*., 2017). Myriads of microbes such as *Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Serratia*, *Campylobacter*, *Aspergillus*, *Penicillium* and viruses have been reported as

contaminants of fomites, and are widely distributed in outdoor and indoor environments (Al-Harbi *et al*., 2017). Some of the fomites where contamination has been reported include telephones (Missri *et al*., 2019), computer keyboards (Smibert *et al*., 2018), healthcare personnel attire and devices (Haun *et al*., 2016), as well as towels (Harrison *et al*., 2003) and handrails (Harrison *et al*., 2003). The spread of pathogenic microorganisms from fomites to humans is driven by factors such as the type of surfaces, type of microorganisms, as well as the hydrophobicity and moisture content of the contact surfaces (Rusin *et al*., 2002; Stephens *et al*., 2019). The cross-contaminations that is associated with fomites-to-human routes have been reported to be significantly ameliorated by physical cleaning (Cogan *et al*., 2002; De Jong *et al*., 2008), sanitization (Rutala *et al*., 2007), inclusive of the use of antimicrobial additives and surface coatings to manufacturing fomites such as door knobs, fabrics and telephones (Kalyon & Olgun, 2001).

Staphylococcus species are known worldwide as a cause of human and animal infections such as bacteremia, wound infections and mastitis (Scott & Bloomfield, 1990; Jones, 2017). They can be differentiated by their ability to produce coagulase, with coagulase-positive staphylococci regarded as more pathogenic than coagulase-negative species (Al-Ghamdi *et al*., 2011). However, the carriage of antibiotic resistance genes amongst strains of the *Staphylococcus* species has been reported to be responsible for increased morbidity and mortality, higher healthcare costs and prolonged hospitalization (Simoes *et al*., 2011; Osei-Sekyere & Mensah, 2020). Even though the transmission of *Staphylococcus* species is mainly via direct human-to-human skin contact route, fomites are also important reservoirs for the spread of *Staphylococcus* species because of their ability to survive on fomites for long periods (Neely & Maley, 2000).

In recent times, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI–TOF MS) is increasingly being used for microbial identification (Reynolds *et al*., 2005; Tsuchida *et al*., 2020). This is because an infinitesimal quantity of samples is often needed by the equipment for analysis, as well as the ease of use of the equipment and the ability to identify either culturable or non-culturable microorganisms. MALDI–TOF MS technique produces microbial peptides fingerprint spectrum that ensures accurate identification of microbial species. Unlike molecular biology, MALDI–TOF MS exhibit no genomic features of the analyzed microbes, thus making this technique a taxonomic tool that does not have direct phylogenetic components (Moore *et al*., 2002).

Materials and methods

A total of 250 swab samples were randomly collected from fomites located at the Igbinedion University Teaching Hospital (IUTH), the Microbiology Laboratory at the Department of Biological Sciences, Igbinedion University, as well as from other areas in Okada town such as the grocery stores and the central market. The fomites that were screened at the sampling locations included the tables, chairs, door handles, keys, switches, handrails and equipment (computers, television, shopping carts and baskets, incubators and microscopes). Sterile cotton swab sticks moistened in 3 ml tryptic soy broth were used to swab the surfaces of the fomites and subsequently transported in ice packs to the Microbiology Laboratory at the Igbinedion University for microbiological analysis.

Bacterial enrichment

Contents of each of the swab sticks used for sample collection were rinsed into the 3 ml tryptic soy broth and the broth incubated at 37^{o}C overnight.

Bacterial isolation and phenotypic identification

A loopful of each of the enriched samples was inoculated onto duplicate mannitol salt agar (MSA) plates by the streak plate technique (Public Health England, 2014). The inoculated Petri dishes were then incubated at 37ºC for 48 hours. After incubation, presumptive staphylococcal colonies on the agar Petri plates were identified by phenotypic tests according to standard methods (Krieg & Holt, 1984). The phenotypic tests that were performed included Gram staining, coagulase, catalase and haemolysis tests.

Bacterial identification by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

Bacterial isolates that were suspected to be staphylococci by phenotypic tests were prepared for mass spectrometry analysis using the formic acid extraction protocol as previously described (Chen *et al*., 2015; Wireko, *et al*., 2021). Pure colonies of suspected staphylococci cultures were suspended in 300 microlitres distilled water, and suspension was subsequently mixed with ethanol (90% v/v) followed by centrifugation at 13000 × g for 2 minutes. Twenty-five microlitres of formic acid (70% v/v) was used to re-suspend the pellets obtained from centrifugation, followed by the addition of 25 microlitres of pure acetonitrile. The mixture was further centrifuged for 2 minutes at 13000 × g. Aliquots of the supernatant (0.5 µl) containing the bacterial proteins were spotted in duplicate onto the MALDI Ground Steel target (Bruker Daltonics, Coventry, UK) and air-dried at room temperature for 5 minutes, followed by overlaying of 1 µl α-Cyano-4-hydroxycinnamic acid matrix solution on each target spot. Analysis of the bacterial isolates was performed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) (Microflex LT, MALDI-TOF MS, Bruker Daltonics, Coventry, UK) in a positive linear mode (2000 to 20000 m/z range). The spectra that were obtained for each of the bacterial cultures were analyzed by MALDI-Biotyper 2.0 software (Bruker Daltonics, Coventry, UK) that compared each spectrum to the reference spectrum in the Bruker Taxonomy Database which identified the best match from the database records. The results were expressed as scores (IS) from 0 – 3. Scores that were less than 1.7 (> 1.7) were not a reliable identification parameter. Genus's identification corresponded to scores that were between 1.70 and 1.99 (1.70 – 1.99), while scores that were greater than or equal to 2.0 (≥ 2.0) corresponded to acceptable species-level identification.

Antibiotic susceptibility test

Suspected staphylococci colonies that were confirmed to be *Staphylococcus* species by MALDI-TOF MS were tested for multidrug resistance by the Kirby Bauer disc diffusion technique according to the guidelines prescribed by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2014). Saline suspension of each pure bacterial colony was incubated and subsequently adjusted to 0.5 McFarland turbidity standards, followed by inoculation on Mueller–Hinton agar plates and the addition of antibiotic discs on the agar surface. The inoculated Petri dishes were incubated at 35°C for 16 hours. After incubation, the inhibitory zone diameter around each of the bacterial colonies was interpreted as resistant, intermediate, or sensitive based on zone diameter interpretive standards (breakpoints) recommended by the Clinical and Laboratory Standards Institute. *Staphylococcus aureus* ATCC 25923 was used as a reference strain for the disc diffusion test. The bacterial isolates were tested against antibiotic discs that included erythromycin (15 µg), gentamicin (10 µg), ampicillin (10 µg), ciprofloxacin (5 µg), pefloxacin (5 µg), ceftriaxone (30 µg), cotrimoxazole (30 µg), cefoxitin (30 µg) and

cefuroxime (30 µg). All the antibiotics employed in this study belonged to five antibiotic classes. Bacterial colonies that exhibited resistance to at least three antibiotics from three different antibiotic classes out of the five different antibiotic classes examined were regarded as multidrug-resistant.

Estimation of multiple antibiotic resistance indices

Multiple antibiotic resistance indices (MAR) of the staphylococci colonies were determined according to the method of Krumperman (Krumperman, 1983). The MAR estimated the risk of acquiring multidrug-resistant staphylococci from the fomites, and was calculated as follows:

$$MAR = \frac{\sum(AR)}{A \times B} \quad (2)$$

MAR is the mean multiple antibiotic resistance index. *AR* is the antibiotic resistance scores of each *Staphylococcus* isolate. *AR* represents the sum of antibiotic classes to which a particular *Staphylococcus* colony exhibited resistance. *A* is the total number of antibiotic classes tested. *B* is the total count of *Staphylococcus* isolates examined. A *MAR* value that was greater than 0.2 (> 0.2) indicated a high-risk source of acquiring multidrug-resistant staphylococci.

Statistical analysis

The prevalence data were estimated as a percentage, with 95% confidence intervals. A Chi-square test was used to compare the presence of fomites in Okada town and their probable contamination with staphylococci. A p-value of less than 0.05 was considered significant in all circumstances. NCSS (version 12) was used to analyze the data.

Results

Characterization of staphylococcal isolates on fomites

Table 1 shows the characterization of suspected staphylococci by MALDI-TOF MS. Each of the presumptive staphylococcal colonies that were subjected to phenotypic tests was suspected to be *Staphylococcus* genus if it was shown to be Gram-positive cocci in clusters, coagulase-variable and catalase-positive. All the 39 staphylococcal isolates obtained from the 250 fomites that were suspected to be staphylococci by the phenotypic tests were all confirmed to be staphylococcal species by the MALDI-TOF MS, corresponding to an overall estimated prevalence of 15.60% (39/250). Speciation by the MALDI-TOF MS indicated that the identified *Staphylococcus* species included *S. haemolyticus*, *S. kloosii*, *S. nepalensis*, *S. saprophyticus*, *S. sciuri*, *S. simulans* and *S. xylosus*. All the confirmed species of staphylococci found on the fomites were negative for the coagulase test and were regarded as coagulase-negative staphylococci. *S. sciuri* were the species that occurred most frequently on the fomites. *S. haemolyticus* and *S. sciuri* were the species that were isolated from switches. *S. kloosii*, *S. nepalensis*, *S. sciuri* and *S. xylosus* were found on door handles. *S. kloosii*, *S. sciuri* and *S. xylosus* were isolated from chairs; while on the handrails; *S. saprophyticus* and *S. sciuri* were the species that were isolated. *S. sciuri* were the only species that were found on the tables, keys, mobile phones and equipment that were examined.

Prevalence of contaminated fomites

The prevalence or likelihood of contamination of the fomites by *Staphylococcus* species is presented in Table 2. The likelihood of contamination of the different types of fomites with *Staphylococcus* species

ranged from 7.81% to 57.14%, with the door handles accounting for the most contaminated fomites and the tables accounting for the least staphylococcal-contaminated fomites. Overall, 15.60% of all the fomites examined in this study were contaminated with *Staphylococcus* species.

Table 1: Characterization of confirmed staphylococci on fomites by MALDI-TOF MS

Identity of confirmed staphylococci on fomites	Prevalence of the identified staphylococci			MALDI-TOF-MS identification scores for the tested staphylococcal isolates	Implicated fomites
	H/N	M (%)	95% CI (%)		
Staphylococcus haemolyticus	1/250	0.40	0.00 – 1.19	2.1	Switch
Staphylococcus kloosii	2/250	0.80	0.00 – 1.91	2.1, 2.2	Door handle and chair
Staphylococcus nepalensis	1/250	0.40	0.00 – 1.19	2.0	Door handle
Staphylococcus saprophyticus	1/250	0.40	0.00 – 1.19	2.0	Handrail
Staphylococcus sciuri	11/250	4.40	1.85 – 6.95	2.0, 2.1, 2.1, 2.2, 2.1, 2.0, 2.1, 2.2, 2.1 2.1, 2.0	Chair, table, handrail, switch, mobile phone, door handle, key, and equipment
Staphylococcus simulans	1/250	0.40	0.00 – 1.19	2.3	Handrail
Staphylococcus xylosus	2/250	0.80	0.00 – 1.91	2.0, 2.2	Chair and door handle
Other confirmed staphylococci	20/250	8.00	4.63 – 11.37	1.7 – 1.9	Door handle, keys, chair, table, handrail, switch, keys, mobile phones and equipment

H: count(s) of confirmed staphylococci on fomites; N: total number of fomites examined; M: mean prevalence; CI: confidence interval of mean

Table 2: Prevalence of fomites contaminated by *Staphylococcus* species

Fomites	Prevalence of staphylococci-contaminated fomites		
	F/N	M (%)	95% CI (%)
Table	5/64	7.81	1.18 – 14.44
Chair	6/61	9.84	2.30 – 17.38
Door handle	8/14	57.14	50.77 – 63.51
Handrail	7/46	15.22	4.72 – 25.72
Key	3/14	21.43	0.00 – 43.73
Mobile phone	6/25	24.00	6.91 – 41.09
Switch	1/9	11.11	0.00 – 32.89
Equipment	3/17	17.65	0.00 – 36.33
All fomites	39/250	15.60	10.39 – 19.21

F: count(s) of confirmed staphylococci on the fomites; N: number of fomites examined; CI: confidence interval of mean; M: mean prevalence

Prevalence of multidrug-resistant staphylococci on the fomites

Tables 3 and 4 show the antibiotic resistance patterns and resistance profile of confirmed *Staphylococcus* species isolated from all the fomites examined in this study. All the staphylococcal colonies obtained from the fomites were resistant to all beta-lactam antibiotics tested. Twenty-three staphylococcal colonies were resistant to all the classes of antibiotics tested out of the 39 tested staphylococcal colonies. The staphylococcal colonies were generally most sensitive to the fluoroquinolone (ciprofloxacin) and aminoglycoside (gentamicin) classes of antibiotics. Twenty-eight staphylococcal colonies were detected to be multidrug-resistant. The 28 multidrug-resistant staphylococci were distributed across 28 different fomites examined in this study. Hence, the likelihood of contamination of all the examined fomites with multidrug-resistant staphylococci was estimated at 11.20% (28/250).

Estimates of MAR associated with contaminated fomites

Overall, the mean multiple antibiotic resistance index (MAR) associated with the contaminated fomites was estimated at 0.79. As indicated by MAR, the contaminated fomites were found to be a potential source of multidrug-resistant staphylococci, with a significant health risk, since the reported MAR exceeded the recommended limit of 0.2.

Association between the fomites and presence of staphylococci

Table 5 shows the contingency table for estimating the association between the fomites and the presence of staphylococci and their multidrug-resistant strains. The chi-square test of independence showed that no significant association was found between the fomites examined in Okada and the presence of either staphylococci ($p = 0.44$) or multidrug-resistant staphylococci ($p = 0.37$).

Table 3: Antibiotic resistance patterns of confirmed *Staphylococcus* species isolated from the fomites

Confirmed staphylococci	Tested colonies	Resistance patterns of the tested antibiotics
Staphylococcus haemolyticus	A1	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus kloosii	B1	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus kloosii	B2	CXM, AM, FOX, CRO, E
Staphylococcus nepalensis	C1	CXM, AM, FOX, CRO, E
Staphylococcus saprophyticus	D1	CXM, AM, FOX, CRO, E
Staphylococcus sciuri	E1	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sciuri	E2	CXM, AM, FOX, CRO, E
Staphylococcus sciuri	E3	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sciuri	E4	CXM, AM, FOX, CRO, PEF, E
Staphylococcus sciuri	E5	CXM, AM, FOX, CRO, E
Staphylococcus sciuri	E6	CXM, AM, FOX, CRO, E
Staphylococcus sciuri	E7	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sciuri	E8	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sciuri	E9	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sciuri	E10	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sciuri	E11	CXM, AM, FOX, CRO, E
Staphylococcus simulans	F1	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus xylosus	G1	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus xylosus	G2	CXM, AM, FOX, CRO, PEF, C
Staphylococcus sp.	H1	CXM, AM, FOX, CRO, E
Staphylococcus sp.	H2	CXM, AM, FOX, E
Staphylococcus sp.	H3	CXM, AM, FOX, CRO, C, GM, E
Staphylococcus sp.	H4	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H5	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H6	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H7	CXM, AM, FOX, CRO, PEF, E
Staphylococcus sp.	H8	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H9	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H10	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H11	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H12	CXM, AM, FOX, CRO, E
Staphylococcus sp.	H13	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H14	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H15	CXM, AM, FOX, CRO, PEF, E
Staphylococcus sp.	H16	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H17	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H18	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H19	CXM, AM, FOX, CRO, PEF, CIP, C, GM, E
Staphylococcus sp.	H20	CXM, AM, FOX, CRO, C

CXM: cefuroxime; AM: ampicillin; FOX: cefoxitin; CRO: ceftriaxone; PEF: pefloxacin; CIP: ciprofloxacin; C: cotrimoxazole; GM: gentamicin; E: erythromycin

Table 4: Antibiotic resistance profile of staphylococci colonies obtained from the fomites

Confirmed staphylococci	K	Prevalence of antibiotic resistance									MR	∑(AR1)	∑(AR2)
		CXM 30 µg F (%)	AM 10 µg F (%)	FOX 30 µg F (%)	CRO 30 µg F (%)	PEF 5 µg F (%)	CIP 5 µg F (%)	C 30 µg F (%)	GM 10 µg F (%)	E 15 µg F (%)			
Staphylococcus haemolyticus	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1	5	153
Staphylococcus kloosii	2	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)	1 (50.00)	1 (50.00)	1 (50.00)	1 (50.00)	2 (100.00)	1	7	
Staphylococcus nepalensis	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	0	2	
Staphylococcus saprophyticus	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	0	2	
Staphylococcus sciuri	11	11 (100.00)	11 (100.00)	11 (100.00)	11 (100.00)	7 (63.64)	6 (54.55)	6 (54.55)	6 (54.55)	11 (100.00)	7	41	
Staphylococcus simulans	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1	5	
Staphylococcus xylosus	2	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)	1 (50.00)	1 (50.00)	2 (100.00)	1 (50.00)	2 (100.00)	2	8	
Other confirmed staphylococci	20	20 (100.00)	20 (100.00)	20 (100.00)	19 (95.00)	15 (75.00)	13 (65.00)	15 (75.00)	14 (70.00)	19 (95.00)	16	83	

K: relative count of confirmed staphylococci colonies; MR: relative count of multidrug-resistant staphylococci colonies; Σ(AR1): relative sum of antibiotic resistance scores; Σ(AR2): overall sum of antibiotic resistance scores; C: cotrimoxazole; GM: gentamicin; E: erythromycin; CXM: cefuroxime; AM: ampicillin; FOX: cefoxitin; CRO: ceftriaxone; PEF: pefloxacin; CIP: ciprofloxacin

Table 5: Contingency table for estimating the association between the fomites and a probable contamination by staphylococci and their multidrug-resistant strains

Sampling locations in Okada metropolis	Number of fomite samples	Presence of staphylococci		Chi square test for staphylococci		Presence of multidrug-resistant staphylococci		Chi square test for MRS	
		Yes	No	X^2	p-value	Yes	No	X^2	p-value
				2.70	0.44			3.16	0.37
IUTH	55	9	46			8	47		
Microbiology Laboratory	51	11	40			8	43		
Grocery stores	48	8	40			5	43		
Central market	96	11	85			7	89		

IUTH: Igbinedion University Teaching Hospital. MRS: multidrug-resistant staphylococci

Discussion

Door handles accounted for most of the contaminated fomites (Tables 1 and 2). Hence the door handles may be the most implicated probable source of acquiring infections in Okada metropolis. In consistence with the current study, Eze *et al.* (2012) and Moore *et al.* (2002) also reported huge bacterial contaminations in door handle swab samples collected from Abuja, Nigeria and United Kingdom, respectively. The high prevalence of contaminated door handles in this study may be due to frequent contact by the human inhabitants and inappropriate hygienic conditions of the environment. Handrails, keys, telephones, as well as equipment such as computers, television, shopping carts and baskets, incubators and microscopes were also found to be significantly contaminated with staphylococci (Table 2), and may also play a substantial role in the spread of infections. Reynolds *et al.* (2005), Al-Ghamdi *et al.* (2011) and Jones (2017) have reported significant biological contamination of shopping carts and baskets with staphylococci. The frequent use of shopping carts and baskets by customers of the grocery stores could be the main reason for contamination since the customers have different hygienic statuses, and upon the handling of shopping carts and baskets, they may transmit pathogenic microbes from their hands to the handles of this shopping equipment, and vice versa.

The survival of the isolated bacterial species on the surfaces of the examined fomites may be ascribed to several factors, some of which may be the nature of the surrounding environment and characteristics of the bacterial species. Many bacteria can use their structures, such as their glycocalyx and flagella, to adhere and survive on fomites and on the hands for several hours (Scott & Bloomfield, 1990).

Transmission of bacterial pathogens may either be through direct or indirect routes. The transmission of bacterial pathogens from contaminated fomites to the hands of humans, and ultimately into the human body by ingestion or inhalation, is a typical example of the indirect pathway. Hence the contaminated fomites examined in this study could indirectly cause severe health problems in humans if adequate hygienic practices are not applied. Typical direct pathways of bacterial pathogen transmission include human to human transmissions. *S. sciuri*, *S. xylosus*, *S. simulans*, *S. haemolyticus*, *S. kloosii* were the main multidrug-resistant *Staphylococcus* species that were found on the examined fomites. Some staphylococci that were identified to the genus level were also found to be multidrug-resistant (Tables 3 and 4). The findings from this study were consistent with those of Smibert *et al.* (2018) who found multidrug-resistant organisms on mobile phones and computer keyboards. The isolated multidrug-resistant bacteria derived from this study exhibited an extremely high prevalence of resistance to all the tested beta-lactam antibiotics (100%). High bacterial resistance to these beta-lactam antibiotics that are frequently used to treat humans calls for concern. However, the bacterial isolates obtained from the present study were most susceptible to the fluoroquinolones (ciprofloxacin) and aminoglycosides (gentamicin) classes of antibiotics. Hence these classes of antibiotics may still provide treatment options for humans that become indirectly infected with multidrug-resistant bacteria mediated by contaminated fomites.

Conclusions

This study revealed that fomites in Okada metropolis were contaminated with staphylococci that have the potential of causing health risks, though not presently at a significant level. Nevertheless, appropriate hygienic measures to mitigate potential staphylococcal cross-contaminations mediated by the contaminated fomites are recommended to avoid potential significant health risks in the future.

Conflicts of interest

There is no conflict of interest in this study.

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(JNASR) Journal of Natural and Applied Sciences Research
Izevbuwa *et al.*, (2025). 1(1): 25-46. Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Prevalence and Risk Factors of High-Risk HPV E6 /E7 Oncoproteins Among Women in Benin-City, Nigeria: Implications for Cervical Cancer Prevention

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Abstract

Cervical cancer remains a major public health issue in Nigeria, primarily driven by persistent infection with high-risk Human Papilloma Virus (HPV) types 16 and 18, particularly through the expression of viral oncoproteins E6 and E7. These oncoproteins are known to disrupt tumor suppressor pathways, contributing to the development of pre-cervical and invasive cervical cancer. This study aimed to assess the prevalence of HPV E6 and E7 oncoproteins and associated risk factors in parts of Benin-City, Edo State. A cross-sectional study design was employed, and 120 cervical samples were collected from consenting participants who met the inclusion criteria. The study population had a mean age of 26.4 years (SD = 2.11), with the majority aged between 18–27 years (51.7%). Most participants were single (65.8%), Christian (76.7%), and students (45.0%), with the highest level of education being secondary (57.5%) and tertiary (26.7%). The overall prevalence of E6/E7 oncoproteins was 4.2%, with the highest positivity observed among participants with early sexual activity ($p = 0.001$). Multiple sexual partners, abnormal vaginal bleeding, and lack of HPV vaccination were found to have significant association with HPV infectivity among the study participants ($p = 0.006$, $p = 0.022$, $p = 0.046$ respectively). Notably, 80% of E6/E7-positive cases were associated with HPV type 16, and 20% with type 18. The findings underscore the importance of E6/E7 oncoprotein screening in identifying women at higher risk of cervical neoplasia and reinforce the importance of integrating molecular-based HPV testing into routine cervical cancer prevention programs. Public health strategies that promote early HPV vaccination and accessible molecular diagnostics are essential to mitigate the burden of cervical cancer in Edo State and similar settings.

Introduction

Cervical cancer remains a major public health concern and ranks as one of the leading causes of cancer-related deaths among women worldwide (Bray *et al.*, 2024). Sub-Saharan Africa, including Nigeria, bears a disproportionate burden of the disease due to limited access to preventive measures such as HPV vaccination and regular cervical screening programs (Jemal *et al.*, 2011). The primary etiological agent of cervical cancer

is Human Papilloma Virus (HPV), a double-stranded Deoxyribonucleic acid (dsDNA) virus from the family *Papillomaviridae* (Ramberg, 2022). Among the over 200 identified HPV types, high-risk strains such as HPV-16 and HPV-18 are responsible for approximately 70% of cervical cancer cases globally (Ferlay *et al*., 2021).

A critical step in HPV-driven carcinogenesis is the persistent expression of two viral oncoproteins, E6 and E7, which play a central role in disrupting cellular tumor suppressor pathways. The E6 protein facilitates the degradation of p53, impairing DNA repair and apoptosis, while E7 inactivates the retinoblastoma protein (pRb), promoting uncontrolled cellular proliferation (Fernandes & De Medeiros Fernandes, 2012). These oncoproteins serve as molecular markers of high-risk HPV infections, making them valuable for early detection and targeted therapeutic strategies.

While high-income countries have made substantial progress in reducing cervical cancer incidence through vaccination and advanced screening techniques, many low- and middle-income countries, including Nigeria, struggle with high disease prevalence (Ferlay *et al*., 2014). In Edo State, factors such as inadequate healthcare infrastructure, cultural misconceptions, poor awareness of HPV, and the lack of access to molecular-based diagnostic tools exacerbate the challenges of early detection and prevention

However, existing screening programs in the region focus primarily on cytological methods, such as Papanicolaou test (Pap smears), which are less effective in identifying high-risk HPV strains and their oncogenic activity (World Health Organization (WHO), 2022). This is because Pap smears detect cellular abnormalities that may result from HPV infection but do not directly identify the virus or determine its specific strain. As a result, they may miss early HPV infections or fail to distinguish between low-risk and high-risk types, limiting their effectiveness in assessing cervical cancer risk (Radosevich, 2012).

Screening for E6 and E7 oncoproteins offers a more sensitive and specific approach to identifying women at risk of developing pre-cervical cancer and cervical cancer. This method not only aids early detection but also provides a critical opportunity to target high-risk populations for timely (WHO, 2006). By evaluating the effectiveness of E6 and E7 oncoprotein screening for pre-cervical cancer in parts of Benin City, Edo State, this study aimed to demonstrate their potential as a diagnostic tool for early detection of pre-cervical cancer and cervical lesions, allowing for timely clinical interventions. This could support the integration of molecular screening into existing cervical cancer prevention programs, improving early intervention strategies and improved patient outcomes (WHO, 2023).

Methodology

A total of 120 cervical samples were collected aseptically using sterile cervical swabs, from women at Faith Mediplex Hospital from August 2024 to October 2024. Sample collection was carried out using sterile cervical swabs and in accordance with recommended clinical standards to ensure patient safety and specimen integrity.

Each participant was positioned in the lithotomy posture, and a sterile speculum was gently inserted to visualize the cervix. Using the Delphi vaginal self-sampler, the sample was collected by inserting the sampler into the vagina and pressing the activation button to absorb the cervical secretions. The sample, which often appeared cloudy and could contain mucus (a normal finding), was then released into a labeled, dry transport tube by depressing the button on the sampler while the sampler was held upright within the tube. Samples were immediately labeled with the participant's identification

code and date of collection to maintain traceability and prevent cross-contamination.

Results

This study included 120 participants, with a mean age of 26.4 years (SD = 2.11). The age distribution showed that the majority of respondents were between 18-27 years (51.7%), followed by 28-37 years (33.3%), 38-47 years (10.0%), and those older than 47 years (5.0%). In terms of marital status, most participants were single (65.8%), while others were married (27.5%), widowed (5.0%), and divorced (1.7%). Religious affiliation was predominantly Christian (76.7%), with smaller proportions identifying as Muslim (20.0%), Traditionalist (0.8%), and other religions (2.5%). Regarding education, 3.3% of participants had no formal education, 12.5% had primary education, 57.5% had secondary education, and 26.7% had tertiary education. Occupational status varied, with 12.5% employed, 32.5% self-employed, 5.8% unemployed, 45.0% students, and 4.2% falling into other occupational categories (Table 1). The prevalence of E6/E7 Oncoproteins of Human Papilloma Virus among participants was found to be 4.2% (5/120) as shown in figure 1.

Table 1. Sociodemographic Characteristics of Participants

Variable	Number (n=120)	Percentage (%)
Age		
18-27 year	62	51.7
28-37 years	40	33.3
38-47 years	12	10.0
>47 years	6	5.0
Marital Status		
Single	79	65.8
Married	33	27.5
Divorced	2	1.7
Widowed	6	5.0
Religion		
Christian	92	76.7
Islam	24	20.0
Traditional	1	0.8
Others	3	2.5
Education		
None	4	3.3
Primary	15	12.5
Secondary	69	57.5
Tertiary	32	26.7
Occupation		
Employed	15	12.5
Self employed	39	32.5
Unemployed	7	5.8
Student	54	45.0
Others	5	4.2

Mean age=26.4±2.11 years.

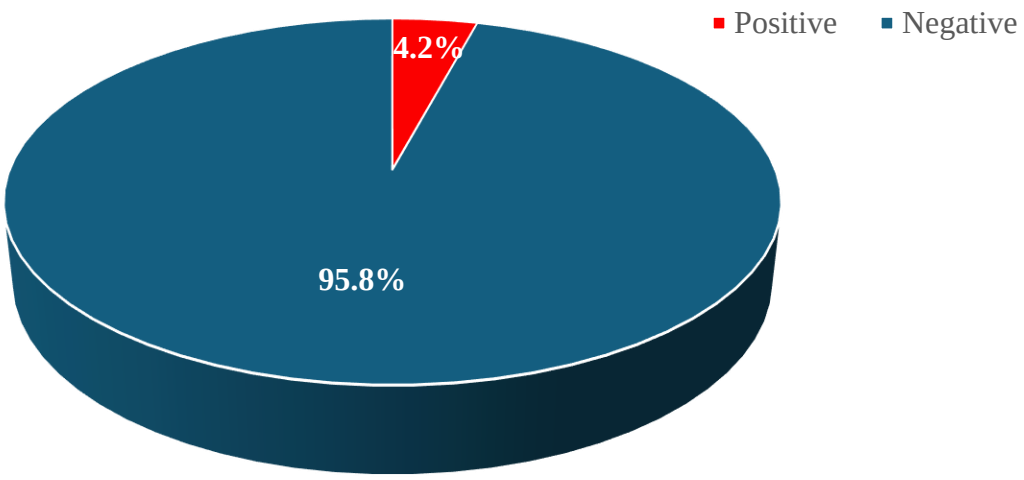

Figure 1. Prevalence of E6/E7 Oncoproteins of Human Papilloma Virus among participants.

Relationship Between Sociodemographic Parameters and Prevalence of E6/E7 Oncoproteins of Human Papilloma Virus

Table 2 shows the relationship between sociodemographic parameters and the prevalence of E6/E7 oncoproteins of Human Papilloma Virus (HPV). The results showed no statistically significant associations between any of the sociodemographic variables and HPV oncoprotein prevalence. Among participants aged 18-27 years, 3.2% were infected, while 5.0% of those aged 28-37 years were infected. No infections were found in participants aged 38-47 years, and 16.7% of those over 47 years were infected. However, the differences were not statistically significant ($\chi^2 = 3.077$, $p = 0.380$). The infection rate was 5.1% among single participants, 3.0% among married participants, and 0% among divorced and widowed participants. This variation was not statistically significant ($\chi^2 = 0.614$, $p = 0.893$). Among Christians, 4.3% were infected, while 4.2% of Muslims were infected. No infections were observed among participants practicing traditional religions or other religions. These differences were not statistically significant ($\chi^2 = 0.181$, $p = 0.981$). The prevalence of infection was 0% among participants with no formal education, 6.7% among those with primary education, 4.3% among those with secondary education, and 3.1% among those with tertiary education. This was not statistically significant ($\chi^2 = 0.501$, $p = 0.919$). The prevalence of infection was 6.7% among employed participants, 0% among self-employed participants, 14.3% among unemployed participants, 5.6% among students, and 0% among those in other occupations. These differences were not statistically significant ($\chi^2 = 4.204$, $p = 0.379$).

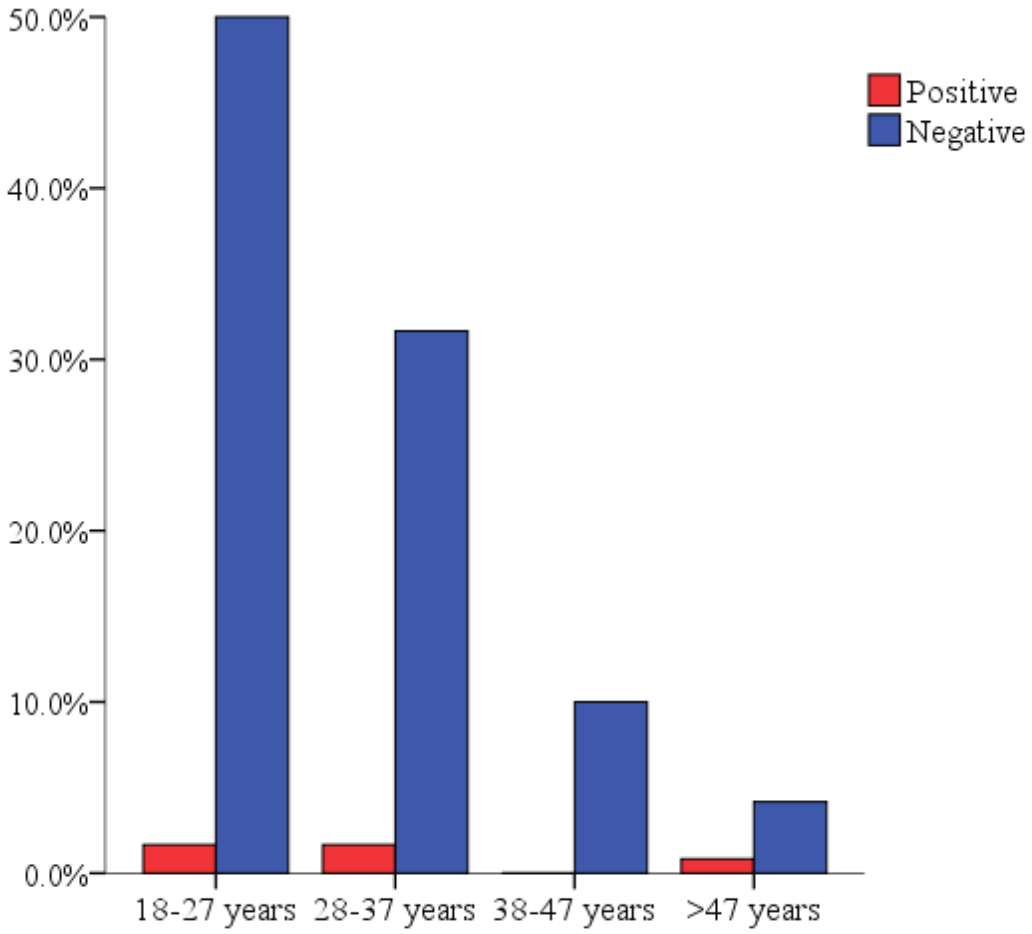

Figure 2. Prevalence of E6/E7 Oncoproteins of Human Papilloma Virus infection among different age groups of participants

Table 2: Relationship Between Sociodemographic Parameters and Prevalence of E6/E7 Oncoproteins of Human Papilloma Virus

Variable	No. Examined (%)	No. Infected (%)	Test Statistics	p value
Age				
18-27 year	62 (51.7)	2 (3.2)	3.077	0.380
28-37 years	40 (33.3)	2 (5.0)		
38-47 years	12 (10.0)	0 (0)		
>47 years	6 (5.0)	1 (16.7)		
Marital Status				
Single	79 (65.8)	4 (5.1)	0.614	0.893
Married	33 (27.5)	1 (3.0)		
Divorced	2 (1.7)	0 (0)		
Widowed	6 (5.0)	0 (0)		
Religion				
Christian	92 (76.7)	4 (4.3)	0.181	0.981
Islam	24 (20.0)	1 (4.2)		
Traditional	1 (0.8)	0 (0)		
Others	3 (2.5)	0 (0)		
Education				
None	4 (3.3)	0 (0)	0.501	0.919
Primary	15 (12.5)	1 (6.7)		
Secondary	69 (57.5)	3 (4.3)		
Tertiary	32 (26.7)	1 (3.1)		
Occupation				
Employed	15 (12.5)	1 (6.7)	4.204	0.379
Self employed	39 (32.5)	0 (0)		
Unemployed	7 (5.8)	1 (14.3)		
Student	54 (45.0)	3 (5.6)		
Others	5 (4.2)	0 (0)		

P<0.05 indicates significance

Prevalence of E6/E7 Oncoproteins of Human Papilloma Virus in association with Cervical Cancer

Table 3 shows the association between the prevalence of E6/E7 oncoproteins of Human Papilloma Virus (HPV) and various risk factors for cervical cancer. Among sexually active participants, 4.4% were infected with HPV oncoproteins compared to 3.4% of those who were not sexually active. However, this difference was not statistically significant (OR = 1.287, 95% CI: 0.138-12.000, p = 0.824). The prevalence of HPV oncoproteins was significantly higher among participants who had their first sexual experience before the age of 18 (25.0%) compared to those aged 18-24 years (2.2%) and those who were over 24 years (0%). This difference was statistically significant (p = 0.001). Participants with more than three sexual partners in the past year had a significantly higher prevalence of HPV oncoproteins (14.8%) compared to those with one partner (0%) or 2-3 partners (2.0%). This difference was statistically significant (p = 0.006). Participants who reported having unprotected sex had a higher prevalence of HPV oncoproteins (9.4%) compared to those who consistently used protection (2.3%). However, this difference was not statistically significant (OR = 4.448, 95% CI: 0.708-27.954, p = 0.085). The prevalence of HPV oncoproteins was 4.7% among those who consistently used protective barriers during sex, compared to 2.9% among those who did not. This difference was not statistically significant (OR = 1.679, 95% CI: 0.181-15.577, p = 0.645). Participants with a history of sexually transmitted infections (STI) had a higher prevalence of HPV oncoproteins (12.5%) compared to those without a history of STIs (3.6%). This difference was not statistically significant (OR = 3.857, 95% CI: 0.379-39.281, p = 0.222).

Table 3: Prevalence of E6/E7 Oncoproteins of Human Papilloma Virus in association with Cervical Cancer

Variable	No. Examined (%)	No. Infected (%)	OR	95%CI	p value
Sexually Active					
Yes	91 (75.8)	4 (4.4)	1.287	0.138-12.000	0.824
No	29 (24.2)	1 (3.4)			
Age of First Sexual Experience					
<18 years	12 (10.0)	3 (25.0)			0.001
18-24 years	89 (74.2)	2 (2.2)			
>24 years	19 (15.8)	0 (0)			
Number of Partners in Past Year					
1	42 (35.0)	0 (0)			0.006
2-3	51 (42.5)	1 (2.0)			
>3	27 (22.5)	4 (14.8)			
Unprotected Sex					
Yes	32 (26.7)	3 (9.4)	4.448	0.708-27.954	0.085
No	88 (73.3)	2 (2.3)			
Consistent Protection Usage					
Yes	85 (70.8)	4 (4.7)	1.679	0.181-15.577	0.645
No	35 (29.2)	1 (2.9)			
History of STI					
Yes	8 (6.7)	1 (12.5)	3.857	0.379-39.281	0.222
No	112 (93.3)	4 (3.6)			

P<0.05 indicates significance

Prevalence of E6/E7 Oncoproteins of Human Papilloma Virus in association with HPV-related Medical History of Participants

Table 4 shows the relationship between the prevalence of E6/E7 oncoproteins of Human Papilloma Virus (HPV) and HPV-related medical history of participants. Participants who reported abnormal vaginal bleeding had a significantly higher prevalence of HPV oncoproteins (16.7%) compared to those without this symptom (2.8%) (OR=7.000, 95% CI: 1.044-46.949, p = 0.022). Participants with persistent pelvic pain had a higher prevalence of HPV oncoproteins (11.1%) compared to those without this symptom (3.6%), the association was not statistically significant (OR = 3.344, 95% CI: 0.333-33.554, p = 0.278). The prevalence of HPV oncoproteins was higher among participants with unusual vaginal discharge (6.9%) compared to those without (3.3%), but this difference was not statistically significant (OR = 2.173, 95% CI: 0.345-13.687, p = 0.398).

Participants who reported painful intercourse had a higher prevalence of HPV oncoproteins (7.9%) compared to those without this symptom (2.4%). However, this association was not statistically significant (OR = 3.429, 95% CI: 0.548-21.432, p = 0.164). None of the participants who had been vaccinated were infected with HPV oncoproteins, compared to 7.4% of those who were not vaccinated. This difference was statistically significant (OR = 1.079, 95% CI: 1.009-1.154, p = 0.046).

Table 4: Prevalence of E6/E7 Oncoproteins of Human Papilloma Virus in association with HPV-related Medical History of Participants

Variable	No. Examined (%)	No. Infected (%)	OR	95%CI	p value
Abnormal vaginal bleeding					
Yes	12 (10.0)	2 (16.7)	7.000	1.044-46.949	0.022
No	108 (90.0)	3 (2.8)			
Persistent pelvic pain					
Yes	9 (7.5)	1 (11.1)	3.344	0.333-33.554	0.278
No	111 (92.5)	4 (3.6)			
Unusual vaginal discharge					
Yes	29 (24.2)	2 (6.9)	2.173	0.345-13.687	0.398
No	91 (75.8)	3 (3.3)			
Painful intercourse					
Yes	38 (31.7)	3 (7.9)	3.429	0.548-21.432	0.164
No	82 (68.3)	2 (2.4)			
Vaccination					
Yes	52 (43.3)	0 (0)	1.079	1.009-1.154	0.046
No	68 (56.7)	5 (7.4)			

P<0.05 indicates significance

Discussion

Human Papillomavirus (HPV), particularly its high-risk strains expressing E6 and E7 oncoproteins, is widely recognized as a significant etiological agent in the development of cervical cancer globally (de Martel *et al.*, 2020). In Nigeria, cervical cancer remains one of the leading causes of cancer related mortality among women, largely attributed to limited HPV vaccination coverage and insufficient public awareness regarding cervical cancer screening practices (Sung *et al.*, 2021; WHO, 2022).

In this research, 120 participants were screened for E6 and E7 oncoproteins of HPV, and the overall prevalence was 4.2%. This prevalence indicates the presence of oncogenic HPV strains in the community and underscores the need for active surveillance and preventive strategies. This also suggests that high-risk HPV strains continue to represent a latent threat, even in regions with low incidence rates. Comparable studies have reported varying prevalence rates across Nigeria. For instance, in a study of 405 women in Awka, Southeastern Nigeria, the prevalence was 19.5%, while estimates ranged from 14.7% in Irun to 21.6% in Okene, 26.3% in Ibadan, and 37.0% in Abuja (Ezechi et al., 2023). A systematic review and meta-analysis of 18 studies likewise estimated a pooled high-risk HPV prevalence of 25% among Nigerian women, with HPV-16 and -18 at 9% and 10%, respectively (Ezechi et al., 2023). Such heterogeneity likely reflects differences in demographic profiles, sexual behavior, laboratory methods, and access to screening and vaccination programmes.

With respect to sociodemographic characteristics of the participants in this study, variables such as age, marital status, religion, education, and occupation did not show any statistical association with the prevalence of E6 and E7 oncoproteins, with p values, 0.380, 0.893, 0.981, 0.919, and 0.379 respectively. This finding is consistent with earlier research, such as that by Akarolo-Anthony *et al*., (2014), which also observed no significant associations between sociodemographic characteristics and HPV infection prevalence. In their study, the lack of correlation was ascribed to the widespread distribution of HPV infection across different demographic groups, suggesting that factors such as age, marital status, and education level may not be the primary determinants of infection risk. The highest infection rate (16.7%) occurred in women above 47 years of age, followed by those in the 28–37 age range (5.0%). This is supported by Piras *et al*. (2011), whose European study showed that persistent HPV infection in older women is likely due to cumulative sexual exposure over time, particularly in populations with delayed access to screening. However, these differences were not statistically significant ($\chi^2 = 3.077$, $p = 0.380$). Single women had a slightly higher prevalence (5.1%) than married women (3.0%), diverging from research associating marriage with prolonged HPV exposure. This may be linked to factors such as lack of parental supervision, peer pressure, economic vulnerability, and limited access to sexual health education and services among single women, which could increase the likelihood of early sexual initiation and multiple sexual partnerships. These conditions have been observed in various settings and may contribute to increased HPV exposure (Narasimhan *et al*., 2015). The high rate of infection among unemployed individuals observed in this study (14.3%) could be attributed to the multiple burdens participants in this category face such as poverty, social isolation, and a lack of consistent healthcare access. Without a source of income or structured routine, unemployed individuals may be more vulnerable to engaging in risky sexual behaviors, including transactional sex, as a means of survival. Additionally, many unemployed individuals may lack the education or awareness necessary to understand the importance of regular HPV screening, vaccination, and safe sex practices, further increasing their susceptibility to infection. These findings correspond with research by Okolo *et al*. (2016), who stated that unemployed women had significantly higher HPV prevalence due to economic instability, reduced healthcare access, and a tendency to engage in survival-driven sexual behaviors.

While these sociodemographic variations may suggest underlying behavioral or access related factors, statistical analysis did not confirm them as significant contributors for HPV infectivity. However, some studies contrast with these observations. For example, Ezechi *et al*. (2017), in a study conducted in Ibadan, South-Western Nigeria, reported a high prevalence (20.3%) for high-risk HPV strains and found a strong correlation with low socioeconomic status alongside contributing factors such as poor personal hygiene, early initiation of sexual activity, multiple sexual partners, and severely limited access to cervical screening programs. The disparity in findings is likely due to the rural setting marked by persistent poverty, overcrowding, and a poorly resourced healthcare system, which may have amplified these risk factors and revealed associations that were inevident in this study. Early sexual activity has been widely recognized as a key risk factor for HPV acquisition and subsequent cervical neoplasia (WHO, 2022). Participants with first sexual activity before 18 years of age had a 25.0% infection rate, which is substantially higher than the 2.2% rate among those aged 18–24, showing statistical significance ($p = 0.001$). These findings correlate with global data linking early sexual activity to increased HPV susceptibility due to cervical immaturity (Gichangi *et al*., 2018). Conforming with findings of Moscicki *et al*. (2020), who studied over 300 adolescent girls in California and concluded that early sexual initiation was associated with higher HPV incidence, emphasizing the importance of delaying sexual interaction through community sensitization, formal sex education in schools, and engagement of parents in adolescent reproductive health awareness. Also, participants with more than three sexual partners in the past year had a higher prevalence (14.8%), compared to those with one partner (0.0%) and those with 2–3 partners (2.0%) ($p = 0.006$). This is in line with research findings of Bruni *et al*. (2022), who conducted a global meta-analysis of over 1 million women across 70 countries in 5 continents and found a strong positive correlation between multiple sexual partners and persistent

HPV infection. This trend was also seen in Nigerian studies such as Chukwuma *et al*. (2018) in Port Harcourt and Efanga *et al*. (2020) in Calabar, who reported higher HPV prevalence among women with multiple partners in cohorts of 200 to 300 participants. Behavioral changes and communication strategies that promote monogamy or fewer sexual partners might be a valuable approach to HPV prevention, as multiple sexual partners increase the likelihood of exposure to different HPV strains and reduce the effectiveness of natural immune responses (Burd, 2021).

Participants who engaged in unprotected sexual intercourse also demonstrated a higher prevalence (9.4%) compared to those who used protective barriers (2.3%), though this was not statistically significant ($p = 0.085$). According to previous research, regular condom use may reduce but not eliminate the risk of HPV transmission (CDC, 2023). Gravitt *et al*. (2017) noted that condoms can effectively reduce viral load and the likelihood of co-infections with other sexually transmitted pathogens, reinforcing their role as an important component in prevention efforts. Likewise, participants with a history of sexually transmitted infections (STIs) had a higher HPV prevalence (12.5%) than those without (3.6%), again corresponding with previous studies suggesting that STIs can compromise mucosal integrity and facilitate HPV infection (LazcanoPonce *et al*., 2021). This finding supports the work of Clifford *et al*. (2013), who conducted a comprehensive meta-analysis examining the co-

infection of HPV with other STIs, particularly *Chlamydia trachomatis* and *Herpes Simplex Virus type 2 (HSV-2)*. Their study proposed that prior or concurrent infections may induce chronic mucosal inflammation, epithelial disruption, and immune modulation, promoting the entry and persistence of HPV.

HPV E6/E7 oncoprotein expression was notably linked to abnormal vaginal bleeding ($p = 0.022$), suggesting this symptom may serve as an early clinical manifestation of cervical epithelial changes. A prevalence of 16.7% was recorded among women who presented with abnormal vaginal bleeding, compared to 2.8% in those without it. This supports the findings of Arbyn *et al.* (2021), who in a European pooled analysis of 15,000 patients identified abnormal bleeding as a significant symptom of early cervical lesions. Although other symptoms such as persistent pelvic pain, unusual vaginal discharge, and painful intercourse were more common among infected individuals, they were not statistically significant ($p = 0.278$), ($p = 0.398$), ($p = 0.164$) respectively. Nevertheless, these findings highlighted the importance of clinical surveillance, particularly in regions with limited access to screening infrastructure. Namujju *et al.* (2021) also established abnormal vaginal bleeding as a frequent warning sign of advanced cervical pathology and should trigger prompt clinical evaluation, including HPV testing and colposcopy. Early detection and timely intervention are essential in halting the progression from HPV infection to cervical intraepithelial neoplasia and, eventually, invasive cervical cancer. The protective benefit of HPV vaccination was clearly demonstrated in this study as none of the vaccinated participants tested positive for E6 or E7 oncoproteins, in contrast to a 7.4% infection rate among unvaccinated individuals. This clear difference reinforces the effectiveness of HPV vaccines, particularly those targeting high risk strains like HPV-16 and HPV-18, in preventing oncogenic infections and their associated lesions. Similar trends have been observed globally, with widespread vaccination contributing to substantial declines in HPV prevalence and related diseases (Drolet *et al.*, 2019; Bruni *et al.*, 2021). However, HPV vaccination coverage in Nigeria remains limited due to barriers such as cost, accessibility, and lack of awareness (Gatumo *et al.*, 2018). These findings highlight the urgent need to strengthen and expand vaccination efforts, particularly among pre-adolescent girls before their sexual debut, to curb the burden of HPV-related conditions.

Conclusion

This study provides important insights into the prevalence and associated risk factors of E6 and E7 oncoproteins of Human Papillomavirus in Benin City, Edo State. The 4.2% prevalence indicates ongoing HPV transmission and the potential for progression to cervical cancer in affected individuals. Significant associations with early sexual activity, multiple sexual partners, abnormal vaginal bleeding, and lack of vaccination emphasize the need for targeted public health interventions. These findings support the efficiency of HPV vaccination, highlight the importance of screening programs and sexual health education in reducing the burden of HPV related diseases and progression to cervical cancer in Nigeria.

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(JNASR) Journal of Natural and Applied Sciences Research
Erifeta *et al.*, (2025). 1(1): 47-59. Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Enzymatic Biomass Transformation; Revolutionizing Renewable Energy

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Abstract

Biomass conversion represents a vital solution in the global transition to sustainable energy systems. This report centers on enzymatic processes for biomass conversion, emphasizing their role in producing renewable energy products such as biofuels, biohydrogen, and bioelectricity. Enzymes, as biocatalysts, enable the efficient breakdown of complex biomass polymers like cellulose, hemicellulose, and lignin into fermentable sugars, which are then utilized in energy production pathways. These processes are characterized by eco-friendliness, operating under mild conditions while avoiding the use of toxic chemicals, making them superior to traditional thermochemical methods. The study explores advancements in enzyme engineering, including thermostable and multi-functional enzymes that enhance reaction rates and scalability. Agricultural residues, forestry byproducts, and organic waste are identified as sustainable feedstocks, reducing dependency on fossil fuels and addressing environmental pollution. Key biochemical pathways such as glycolysis, fermentation, and methanogenesis are discussed, showcasing their contributions to energy generation and carbon footprint reduction. Despite challenges like high enzyme production costs and biomass recalcitrance, innovations in synthetic biology, immobilized enzyme technologies, and integrated biorefineries are paving the way for cost-effective applications. Case studies and global initiatives illustrate the economic viability and environmental benefits of enzymatic biomass conversion. By aligning with international climate action policies and advancing technological innovations, enzymatic biomass conversion is positioned as a cornerstone in achieving energy security, mitigating climate change, and fostering a circular bioeconomy. This report emphasizes the urgent need to harness enzymatic technologies for a sustainable and renewable energy future.

KEYWORDS: **Biomass**, **Enzymes**, **Renewable Energy**, **Biofuels**, **Waste Materials**

Introduction

The increasing demand for alternative and sustainable sources of energy, chemicals, and materials has led to the exploration of biomass conversion technologies (Demirbas, 2007). Biomass, derived from organic materials such as plants, agricultural residues, and waste, offers a renewable and abundant feedstock for the production of biofuels, biochemicals, and biomaterials (Tripathi *et al*., 2016). The utilization of biomass not only addresses environmental concerns associated with fossil fuels but also promotes the development of a circular bioeconomy (Cherubini, 2010). Biomass conversion involves various processes, including thermochemical, biochemical, and physicochemical methods, each tailored to specific feedstocks and desired products (Mohan *et al*., 2006). Thermochemical processes, such as pyrolysis, gasification, and combustion, involve the application of heat to decompose biomass into valuable products like bio-oil, syngas, and biochar (Bridgwater, 2012). Biochemical conversion, on the other hand, relies on enzymatic and microbial actions to transform biomass into bioethanol, biogas, and other bio-based chemicals (Sánchez & Cardona, 2008).Recent advancements in biotechnology have further enhanced biomass conversion efficiency through genetic engineering of microorganisms and development of robust enzymes capable of degrading complex biomass components (Mosier *et al*., 2005). Moreover, the integration of biomass valorization approaches enables the extraction of high-value compounds, thereby improving the overall economics and sustainability of biorefineries (Clark & Deswarte, 2015).

The conversion of biomass into value-added products involves thermochemical, biochemical, and physicochemical processes, each with distinct advantages depending on the feedstock and desired end-products (Bridgwater, 2012; Mohan *et al*., 2006). Among these, biochemical processes, such as enzymatic hydrolysis and microbial fermentation, are particularly attractive due to their mild operational conditions and potential for specificity (Alvira *et al*., 2010). Biotechnology has significantly advanced the efficiency of biomass conversion by improving enzyme formulations and microbial strains capable of breaking down complex lignocellulosic structures (Sánchez & Cardona, 2008; Mosier *et al*., 2005). These innovations have enhanced the yield and cost-effectiveness of producing biofuels and biochemicals from renewable sources. Furthermore, the valorization of biomass into industrially relevant compounds aligns with global sustainability goals and circular economy principles (Clark & Deswarte, 2015; Cherubini, 2010). Thermochemical conversion processes, including pyrolysis, gasification, and combustion, decompose biomass at high temperatures to produce syngas, bio-oil, and biochar (Bridgwater, 2012). Pyrolysis, which occurs in the absence of oxygen, produces liquid bio-oil and solid biochar, both of which can be used as fuel or soil amendments. Gasification partially oxidizes biomass to create a combustible gas mixture that can be utilized for power generation. Combustion, the most direct method, involves burning biomass in the presence of oxygen to generate heat or electricity (Mohan *et al*., 2006). Biochemical conversion, on the other hand, utilizes microbial or enzymatic processes to convert biomass into biofuels like biogas, ethanol, and butanol. Anaerobic digestion, for instance, involves the breakdown of organic material by microorganisms in the absence of oxygen to produce biogas primarily composed of methane and carbon dioxide (Appels *et al*., 2008). Fermentation processes, often used with carbohydrate-rich biomass, convert sugars into ethanol using yeast strains (Sánchez & Cardona, 2008).

Cellulose, hemicellulose, and lignin are major components of lignocellulosic biomass. Effective biomass conversion, particularly for biofuel production, often requires pretreatment to break down the complex. Therefore, the current study seeks to investigate the efficiency, scalability and environmental impact of enzyme based processes for biomass conversion into renewable energy.

Methods

This study investigates innovative bioprocesses that uses enzymes to convert biomass into renewable energy. Electronic searches of the literature, primarily in databases such as PubMed, Google Scholar, Scopus, and ScienceDirect were used to gather published articles for the development of the manuscript. Keywords such as biomass conversion, bioenergy and biofuels were used. A total of 50 scholarly items, including research articles, reviews, books, and other publicly accessible internet sources, were returned by the search procedure. The shortlist included about 15 items and the criteria for selection was based on the use of enzyme for biomass conversion. Due to the scarcity of contemporary studies and their relevance to the chosen topic, articles published before 2010 were also considered. The selected articles were thoroughly studied and critically analyzed for this study.

Result/discussion

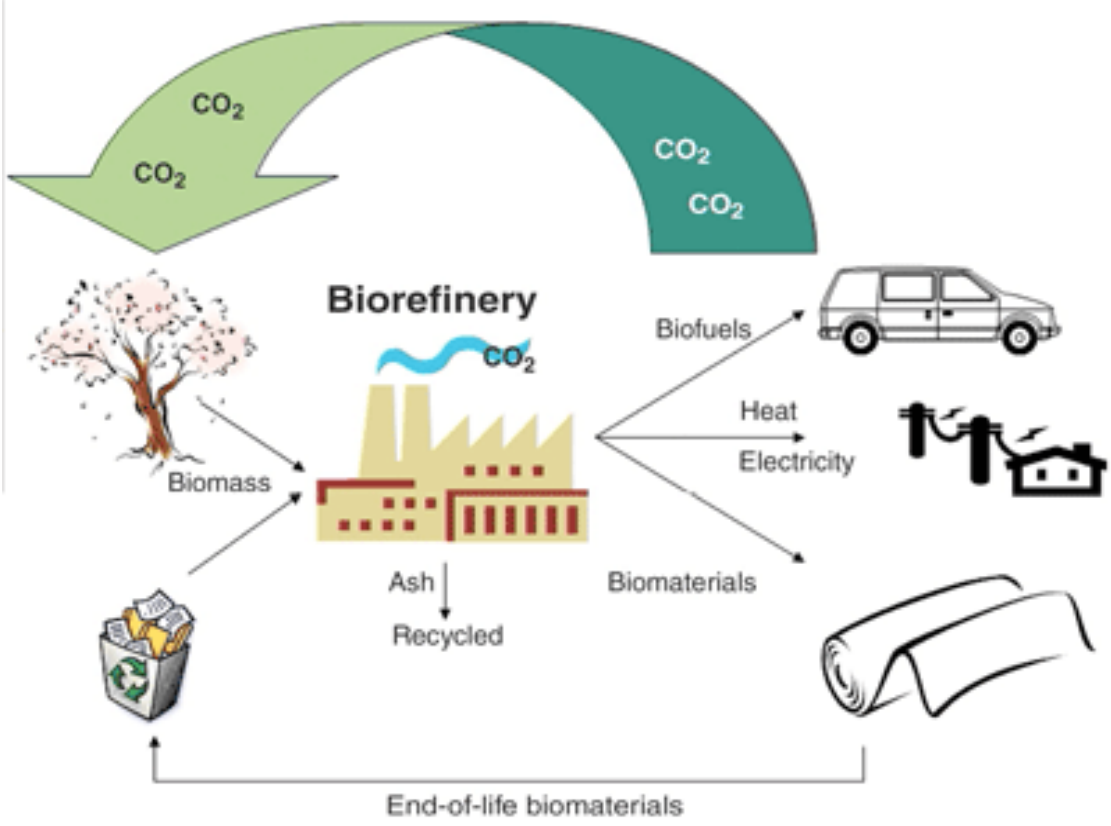
Climate Change and the Need for Renewable Energy

Climate change remains one of humanity's most pressing challenges. The increasing concentration of greenhouse gases, primarily from burning fossil fuels, has led to rising global temperatures, extreme weather events, and significant biodiversity loss (IPCC, 2021). A sustainable energy transition is essential to mitigate these effects.

Renewable energy

Renewable energy refers to energy derived from natural processes that are continuously replenished, such as sunlight, wind, water, and biomass. Unlike fossil fuels, which are finite and contribute to greenhouse gas emissions, renewable energy sources are sustainable and environmentally friendly, making them essential in combating climate change (IEA, 2022). Biomass includes plant materials, agricultural and forestry residues, municipal waste, and algae. It is widely available across different regions, making it an attractive and equitable energy source. Biomass energy can be harnessed through direct combustion, anaerobic digestion, biochemical conversions, and thermochemical processes (IEA, 2022).

However, lignocellulosic biomass primarily composed of cellulose, hemicellulose, and lignin is naturally resistant to microbial degradation, a feature known as biomass recalcitrance. Effective conversion requires innovative treatment strategies to overcome this challenge.



(Extracted from Zafar, 2023)

Enzymes in biomass conversion

Role of Enzymes in Biomass Conversion

Enzymes are biological catalysts that accelerate chemical reactions without being consumed. In biomass conversion, they are essential for breaking down complex organic materials into simpler molecules, enabling the production of biofuels, biogas, and other renewable energy products.

Their specificity, efficiency, and eco-friendliness make them a cornerstone of modern biomass conversion technologies (Bhat & Bhat, 1997)).

Types of Biomass Components Targeted by Enzymes

- Biomass is composed of complex polymers that enzymes act upon:
- Cellulose: A polysaccharide forming the primary structural component of plant cell walls.
- Hemicellulose: A heterogeneous polysaccharide surrounding cellulose fibers.
- Lignin: A complex aromatic polymer providing rigidity and resistance to degradation.

(Kumar *et al*., 2008).

Key Enzymes in Biomass Conversion

Enzyme	Function	Subtypes	Application	References
Cellulases	Break down cellulose into glucose, which can be fermented into biofuels.	• Endoglucanases: Cleave internal bonds in cellulose chains. • Exoglucanases (Cellobiohydrolases): Remove cellobiose units from chain ends. • β-Glucosidases: Hydrolyze cellobiose into glucose	Ethanol and biogas production	(Lynd *et al.*, 2002).
Hemicellulases	Degrade hemicellulose into pentoses (e.g., xylose) and hexoses	• Xylanases: Break down xylan into xylooligosaccharides. • Arabinofuranosidases: Remove arabinose side chains.	Enhancing fermentable sugar yield.	(Wong *et al.*, 1988).
Ligninases	Degrade lignin, a major barrier to accessing cellulose and hemicellulose.	• Laccases: Oxidize lignin and phenolic compounds. • Peroxidases (e.g., lignin peroxidase, manganese peroxidase): Break aromatic structures in lignin	Delignification for biofuel production.	(Martinez *et al.*, 2005).
Auxiliary Enzymes: • Pectinases • Amylases • Lipases	Break down pectin in plant cell walls. Convert starches into fermentable sugars. Hydrolyze fats and oils into glycerol and fatty acids, aiding biodiesel production			(Kuhad *et al.*,. 2011)

Enzymatic Biomass Conversion Processes

1. Enzymatic Hydrolysis: Converts cellulose and hemicellulose into fermentable sugars using cellulases and hemicellulases. Mild conditions (e.g., pH 4.5-5.5, 45-50°C) make the process eco-friendly (Sun & Cheng, 2002).

2. Pretreatment Enhancements: Ligninase enzymes are used during pretreatment to remove lignin and improve enzyme

accessibility to cellulose. Pretreatments include steam explosion, dilute acid hydrolysis, and alkali treatments. (Mosier *et al*. 2005).

3. Fermentation: Sugars produced from enzymatic hydrolysis are fermented into biofuels (e.g., ethanol, butanol) by microorganisms.

4. Biogas Production: Enzymes enhance anaerobic digestion efficiency by breaking down complex polymers into smaller, digestible molecules.

Biochemical Pathways in Biomass Conversion

Biomass conversion into energy products involves several biochemical pathways:

- Glycolysis: Breaks down glucose to pyruvate, yielding ATP and NADH (Berg *et al*., 2002).
- Fermentation: Converts pyruvate into products like ethanol or butanol under anaerobic conditions (Ingledew, 1999).
- Methanogenesis: Archaea convert carbon dioxide and hydrogen into methane during anaerobic digestion (Weiland, 2010).
- Transesterification: Conversion of lipids into biodiesel using chemical or enzymatic catalysts (Ma & Hanna, 1999).

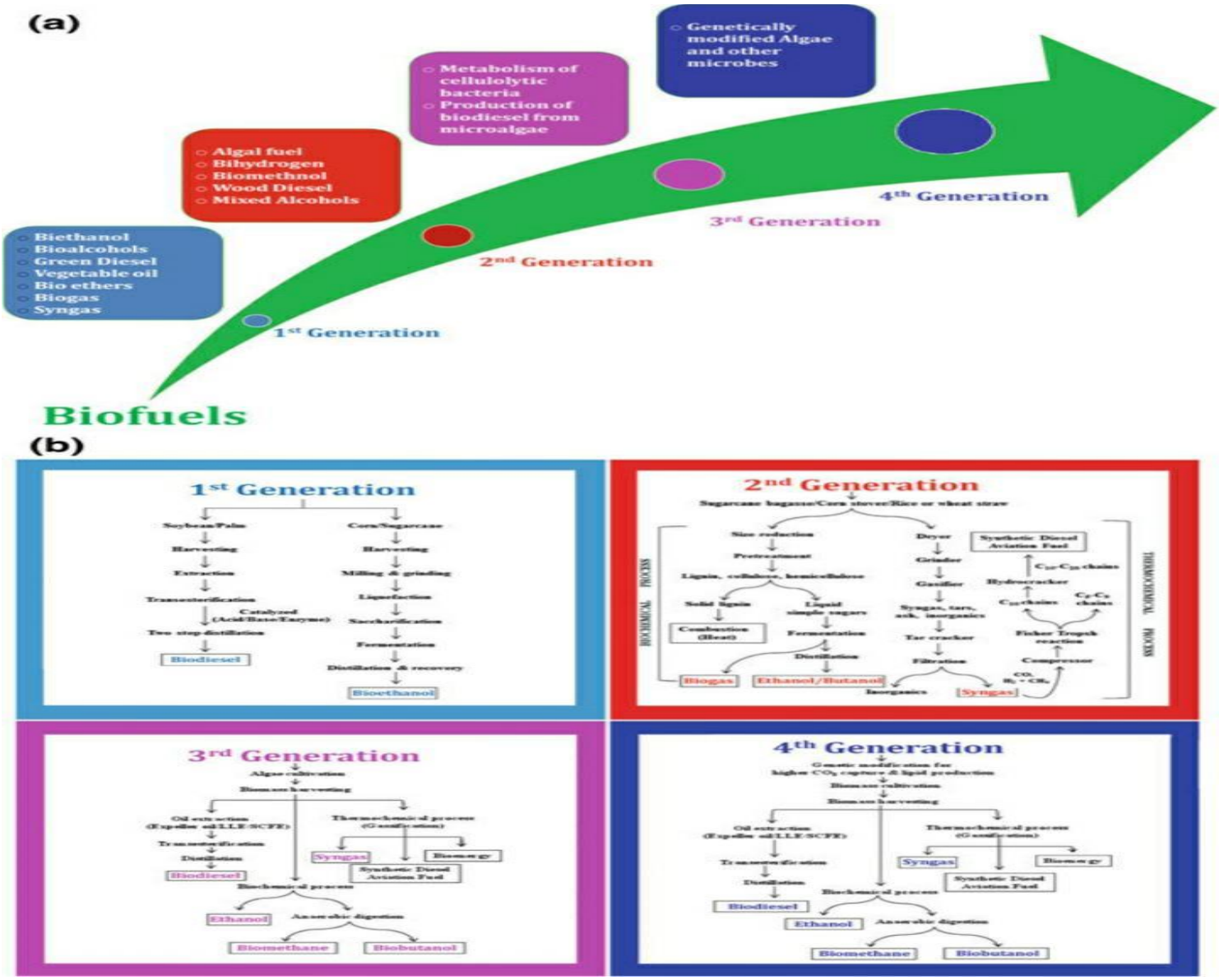
Each pathway offers distinct energy products and can be optimized through biotechnological innovations.

Biofuel Production from Biomass

Biofuels represent a major product of enzymatic biomass conversion. They are categorized into four generations based on feedstock type and production technology (Naik *et al*., 2010):

- First-Generation Biofuels: Derived from food crops such as corn and sugarcane (e.g., corn ethanol, sugarcane ethanol).
- Second-Generation Biofuels: Produced from non-food biomass like agricultural residues, forestry waste, and energy grasses (e.g., cellulosic ethanol).
- Third-Generation Biofuels: Focus on algae and other microorganisms capable of high-yield biofuel production.
- Fourth-Generation Biofuels: Involve genetically engineered organisms that produce biofuels directly from CO_2, often integrated with carbon capture technologies (Chisti, 2007).

Among these, cellulosic ethanol has gained significant attention due to its ability to utilize agricultural and forestry residues, minimizing food-versus-fuel conflicts. Biodiesel is another important product, made through transesterification of fats and oils. While traditional biodiesel production uses chemical catalysts like NaOH or KOH, enzymatic biodiesel production uses lipases, offering a more environmentally friendly alternative (Meher *et al*., 2006). Overall, enzymatic conversion pathways offer several advantages of Improved selectivity, Lower energy consumption and Minimal generation of side-products.



Source: Kour *et al*., 2023)

Case studies and global initiatives

Case studies

1. DuPont Cellulosic Ethanol Plant (USA)

DuPont established one of the world's largest cellulosic ethanol plants in Nevada, Iowa. The facility utilized corn stover (agricultural waste) as a feedstock and focused on enzymatic hydrolysis technologies (DuPont, 2016). However, high enzyme costs and feedstock logistics posed challenges, leading to the eventual sale of the plant.

2. Novozymes and Raízen (Brazil)

Novozymes partnered with Raízen to produce second-generation ethanol from sugarcane residues (bagasse and straw). This collaboration highlights Brazil's leadership in bioethanol production and shows the viability of enzymatic biomass conversion at commercial scale (Novozymes, 2019).

3. LanzaTech Gas Fermentation (China)

LanzaTech's technology captures industrial waste gases (CO and CO_2) and biologically converts them into bioethanol using specially engineered microorganisms. This approach not only reduces industrial carbon emissions but also generates valuable biofuels, embodying circular economy principles (LanzaTech, 2020).

International Policies and Programs

Global initiatives that support biomass and bioenergy development include:

- Paris Agreement (2015): Commitments to reduce carbon emissions, fostering renewable energy transitions (UNFCCC, 2015).
- EU Renewable Energy Directive (RED II): Mandates minimum renewable energy targets for member states (European Commission, 2020).
- US Renewable Fuel Standard (RFS): Requires increasing volumes of renewable fuel to be blended with transportation fuel.

Such frameworks encourage investment, innovation, and scale-up of enzymatic biomass conversion technologies.

Future trends and innovations

The enzymatic biomass conversion sector is evolving rapidly, driven by technological advancements and the urgent need for sustainable energy alternatives. Several emerging trends are shaping the future landscape:

1. Development of Thermostable and Multifunctional Enzymes

Enzymes traditionally operate best under mild temperatures and pH ranges. However, industrial biomass conversion demands robustness. Recent research focuses on engineering thermostable, alkaline-tolerant, and multifunctional enzymes that can withstand industrial processing conditions without losing activity (Liu *et al*., 2022).

Multifunctional enzymes, capable of acting on multiple substrates simultaneously (e.g., cellulose and hemicellulose), are reducing the need for enzyme cocktails and simplifying bioprocesses.

2. Integrated Biorefineries

The concept of the integrated biorefinery mimics petroleum refineries but uses biomass as feedstock. Integrated biorefineries produce multiple products — biofuels, bioplastics, biochemicals, and bioenergy — maximizing value from each ton of biomass (IEA Bioenergy, 2023).

Key components include:

- Pretreatment units.
- Enzymatic hydrolysis reactors.
- Fermentation systems.
- Downstream product recovery systems.

This diversification enhances economic viability and supports circular economy principles.

3. CRISPR and Synthetic Biology for Microbial Engineering

CRISPR-Cas9 and other genome-editing tools enable the creation of designer microbes tailored for efficient biomass conversion. These microorganisms can:

- Secrete improved enzymes.

- Tolerate inhibitors produced during pretreatment.
- Directly ferment pentose and hexose sugars to biofuels (Zhang *et al*., 2022).

Synthetic biology approaches also allow for the assembly of artificial metabolic pathways, further optimizing conversion yields and process efficiencies.

4. AI and Digitalization in Biomass Processing

Artificial Intelligence (AI) and Machine Learning (ML) are transforming how biomass conversion processes are designed and optimized:

- Predicting optimal enzyme combinations.
- Modeling fermentation kinetics.
- Automating biorefinery operations (Patel *et al*., 2023).
- Digital twins — virtual replicas of biorefineries — allow real-time process monitoring, troubleshooting, and efficiency improvements.

5. Decentralized Biomass Energy Systems

Decentralized, small-scale biomass systems offer localized energy solutions, especially in rural and underserved regions. By installing community-based anaerobic digesters, mini-biorefinery units, and gasifiers, communities can achieve energy independence, improve waste management, and foster economic development (Kumar & Sharma, 2022).

Conclusion

Enzymatic biomass conversion stands at the forefront of global efforts to transition toward sustainable, renewable energy systems. Its eco-friendly attributes, coupled with technological advancements in enzyme engineering, bioprocessing, and system integration, make it a cornerstone solution to address climate change, fossil fuel depletion, and rural development challenges. While economic and logistical hurdles remain, the future of enzymatic biomass conversion appears promising. Increased investment in research, supportive public policies, and interdisciplinary collaborations are essential to unlocking its full potential (Chisti, 2007; Demirbaş, 2009). Harnessing the full power of enzymes in biomass conversion will diversify the world's energy portfolio and also contribute significantly to achieving the United Nations Sustainable Development Goals (SDGs), particularly those related to clean energy, climate action, and sustainable industrialization.

Recommendations

To fully harness the potential of enzymatic biomass conversion and promote a sustainable energy future, the following strategies are recommended:

- Invest in Enzyme Research and Development

Funding should prioritize the discovery, engineering, and optimization of robust enzymes that can operate under industrial conditions (Liu *et al*., 2022).

Emphasis should be placed on producing enzymes with broad substrate specificity and resistance to inhibitors formed during biomass pretreatment.

- Promote Sustainable Biomass Sourcing

Biomass feedstocks must be sourced sustainably to avoid deforestation, soil degradation, and food-versus-fuel conflicts.

Policies should support the use of agricultural residues, forestry waste, and non-edible crops.

- Develop and Expand Integrated Biorefineries

Governments and private sectors should invest in the development of integrated biorefineries capable of producing multiple high-value products.

This approach maximizes economic returns while minimizing environmental footprints.

- Support Cost-Reduction Strategies

Cost remains a barrier to commercialization. Innovations like enzyme immobilization, consolidated bioprocessing (CBP), and on-site enzyme production should be encouraged (Sun & Cheng, 2002).

- Strengthen Policy Frameworks

Governments should enact supportive policies such as tax credits, feed-in tariffs, renewable portfolio standards, and carbon pricing mechanisms to incentivize biomass conversion investments (European Commission, 2020).

- Foster Public-Private Partnerships

Collaborations between universities, industries, and governments can accelerate research translation, commercialization, and public acceptance.

- Educate and Raise Awareness

Public education campaigns and curriculum integration at tertiary levels are necessary to build awareness of biomass energy's potential and benefits.

- Advance Decentralized Rural Energy Systems

Developing decentralized biomass conversion units for rural communities can provide localized energy, improve livelihoods, and reduce urban migration pressures (Kumar & Sharma, 2022).

- Monitor Environmental Impacts

Life cycle assessments (LCA) should be conducted for all biomass projects to ensure sustainability across environmental, social, and economic dimensions (Naik *et al*., 2010).

- Invest in Emerging Technologies

Cutting-edge fields like synthetic biology, CRISPR genome editing, and AI-driven process optimization should be leveraged to revolutionize biomass conversion pathways.

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(JNASR) Journal of Natural and Applied Sciences Research
Erifeta *et al.*, (2025). 1(1): 60-75. Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Biomass Waste Valorization: Exploring New Frontiers as Biochemists.

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Abstract

Biomass waste valorization focuses on the conversion of biological waste materials (like agricultural residues, food waste, or forestry by-products) into valuable products such as biofuels, biochemicals, and bio-based materials. It is a sustainable and specific approach to waste valorization that is based on the production of Value-Added Biochemicals. It explores eco-friendly biochemical methods for the valorization of biomass waste into commercially useful compounds. These efforts are essential for environmental sustainability and socio-economic development, as a healthy environment provides resources and ecosystem services while ensuring environmental health. This review highlights advancements in maximizing biomass utilization in waste-to-wealth processes, offering significant waste reduction potential. Innovations in biochemical engineering have enabled the conversion of organic waste into valuable products beyond biofuels, including biogas, bioelectricity, biochar, syngas, bioplastics, bio-based chemicals, animal feed, biofertilizers, green hydrogen, bio composites, pellets, liquid fertilizers, essential oils, and activated carbon etc. Techniques like anaerobic digestion, fermentation, and hydrothermal liquefaction are at the forefront, providing sustainable methods to convert biomass into these diverse resources. A key challenge in waste-to-wealth conversion is optimizing product yield while minimizing waste and environmental impacts. Advances in enzyme engineering and microbial biotechnology have significantly improved process efficiency. For example, engineered microorganisms enhance the conversion of biomass into bioplastics and bio-based chemicals, improving biomass utilization. Furthermore, the production of green hydrogen and biocomposites offers promising alternatives to non-renewable resources, supporting a circular economy. Integrating waste-to-wealth systems with existing waste management infrastructure provides dual benefits: reducing waste volumes and generating renewable resources. This approach addresses waste disposal, reduces greenhouse gas emissions, and supports global sustainability goals, contributing to a cleaner, more resource-efficient future while diversifying sustainable materials and energy options from biomass.

Keywords: Waste valorization, Biotechnology, Bioresources, Biofuels, Sustainability

Introduction

The global surge in industrialization, urbanization and population growth has escalated waste generation, with municipal solid waste (MSW) projected to increase from 2.1 billion metric tons in 2023 to 3.8 billion by 2050, posing existential threat to ecosystems and human health (UNEP, 2024; Waheed *et al*., 2022; Reddy *et al*., 2023). The global direct cost of waste management was an estimated USD 252 billion in 2020. Poor health and climate change from poor waste disposal practices could raise the cost to USD 361 billion (UNEP). (2024). Industrial and agricultural by-products such as plastics and crop residues add to this burden (Olatunji *et al*.,

2024). Conventional waste management methods such as landfilling and incineration, contribute to greenhouse gas emissions, soil and water contamination as well as biodiversity loss, while waste complexity and associated costs has made waste management difficult thereby exacerbating environmental degradation (Alao *et al*., 2022; Anyaegbunam, 2016). Transitioning to a circular economy which emphasizes resource efficiency and waste reintegration is critical for sustainability (Ferreira *et al*., 2024). Waste valorization redefines waste as a resource for producing energy, materials, and bio-based products thereby reducing ecological impact and fostering innovation in bioprocess engineering and microbial biotechnology (Rene *et al*., 2023). This approach, including biofuel production from agricultural residues and municipal solid waste supports energy security, economic growth and job creation in green technologies potentially unlocking $4.5 trillion by 2030 (Arancon *et al*., 2013; Ferreira *et al*., 2024). Hence, the present study aims to explore and evaluate innovative bioprocesses that biochemists can employ to convert biomass waste into high-value bioproducts.

Methods

This study evaluates innovative bioprocesses that biochemists can employ to convert biomass waste into high-value bioproducts. Electronic searches of the literature, primarily in databases such as PubMed, Google Scholar, Scopus, and ScienceDirect were used to gather published articles for the development of the manuscript. Keywords such as biomass waste valorization**,** Bioresources, Biofuels, Sustainability, bioenergy and biopolymer were used. A total of 100 scholarly items, including research articles, reviews, books, and other publicly accessible internet sources, were returned by the search procedure. The shortlist included about 20 items that were published from 2015. Due to the scarcity of contemporary studies and their relevance to the chosen topic, articles published before 2015 but not before 2010 were also chosen. The selected articles were thoroughly studied and critically analyzed for this study.

Results/Discussion

Bioenergy Production from Waste

The global rise in energy demand, fossil fuel depletion, carbon emission and environmental concerns from waste drive the need for sustainable waste-to-energy (WTE) technologies and circular economy solutions (Mishra *et al*., 2023). Organic waste such as sugarcane bagasse, rice paddy straw and cattle manure has been used to produce biogas, biodiesel and bioethanol aiding waste management (Mishra *et al*., 2023). Plastic and solid biomass waste can be co-pyrolyzed into biofuels and value-added products offering an eco-friendly waste management solution (Wang *et al*., 2021). WTE technologies convert waste into biogas (CO_2, CH_4), liquid biofuels (biodiesel, ethanol), and syngas ($CO+H_2$) for electricity generation. Biodiesel is produced through transesterification, achieving 99.6% pure fatty acid methyl ester from waste cooking oil using supercritical methanol and KOH (Mishra *et al*., 2023). Agricultural waste like rice straw and sugarcane bagasse has been hydrolyzed to produce acetone, butanol and ethanol using Clostridium species. While anaerobic digestion of municipal solid waste yields biogas (Mishra *et al*., 2023).

Production Of Biopolymers, Biochemicals and Enzymes from Waste

The production of biopolymers and biomaterials from organic waste through processes like fermentation, enzymatic hydrolysis and anaerobic digestion supports the circular economy (Jain *et al*., 2022). Biopolymers such as polyhydroxyalkanoates (PHA) and polyhydroxybutyrate (PHB) derived from food waste are biodegradable. When used in the production of

bioplastics, degrade into CO_2 and water within months after they are buried, thus reducing plastic and food waste (Capanoglu *et al*., 2022). Biopolymers are used in critical applications in industries like medicine, cosmetics, pharmaceuticals, food industries, water treatment, biosensor development, plastics industries and textiles due to their biodegradability and biocompatibility (Ranganathan *et al*., 2020). Bio-composites from wastes like eggshells, poultry waste, and avocado peel have been used in food preservation and packaging because of their antioxidant and antimicrobial properties (Oluba *et al*., 2024). Aguiló-Aguayo *et al*., (2024), have reported the extraction of Microfibrillated cellulose (MFC) from agroindustrial byproducts. Organic acids such as lactic, succinic, citric have been produced from food waste fermentation and this is influenced by waste composition (Capanoglu *et al*., 2022). Enzymes like α-amylase, glucoamylase, and lipase have been recovered from food waste through fermentation reducing production costs and environmental impact while maintaining high specificity and yield (Capanoglu *et al*., 2022).

Production of Phytochemicals

The demand for plant-derived phytochemicals is growing due to their use in allopathic medicine, either as direct extracts or purified compounds for drug development despite synthetic alternatives (Saravanan *et al*., 2021). Plant wastes are generated in large quantities resulting in pollution. These plant wastes are found to possess therapeutic properties, for example dried mango leaves (Mangifera indica) are rich in antioxidants, antiviral, anti-diabetic, and antitumor compounds like mangiferin (Imran *et al*., 2017). Phenolic compounds, valued for their antioxidant properties interact with lipids, DNA, and proteins to form natural therapeutic agents and have been extracted from agro-industrial waste (e.g., vegetables, fruits, shells, leaves) as tannins, flavonoids, alkaloids, and anthocyanins (Singh *et al*., 2018; Arun *et al*., 2020). Tomato processing waste such as seeds and peels is a source of bioactive compounds like sterols, terpenes, and polyphenols, while coffee production waste contains tannins and phenolic compounds (Navajas-Porras *et al*., 2025).

Production of Biofertilizers

Plants require nutrients like nitrogen, potassium, and phosphorus for growth, which are depleted from soil after harvest and replenished naturally or through fertilizers (Varjani *et al*., 2019). Chemical fertilizers, commonly used to boost crop yield and pest control have been reported to negatively impact both soil microbes and plants. However, organic fertilizers enhance soil's physical, chemical, and biological properties by microbial breakdown of organic compounds (Varjani *et al*., 2021). Agro-industrial waste rich in nitrogen, phosphorus, and potassium, improves soil fertility and crop yield, offering a sustainable alternative to chemical fertilizers with reduced environmental impact (Mohanty *et al*., 2021; Adesra *et al*., 2021). Biofertilizers formulated from oil palm (Elaeis guineensis) empty fruit bunches and plant growth promoting microbes have been reported (Mahmud & Chong, 2021).

source of bio-waste, treatment approaches and its bio-products and bioenergy

Adapted from Jain *et al*., 2022

Pathways for waste conversion

Biochemical conversion involves the microbial decomposition of biodegradable organic waste by bacteria and fungi occurring in the presence or absence of oxygen to produce products like compost, biogas, biofuels, antibiotics, biofertilizers, and biochemicals. This process is ideal for wastes with high biodegradable organic matter and moisture content which support microbial activity. Key pathways include anaerobic digestion, bio-fermentation, and enzymatic hydrolysis.

Anaerobic Digestion

Anaerobic digestion (AD) is a widely adopted process for managing organic waste, reducing environmental impact and producing energy particularly methane, while supporting sustainable development. In Europe, AD handles 25% of biological treatment with 244 plants and an 8-million-ton capacity (Adekunle & Okolie, 2015). It degrades organic matter under anaerobic conditions using bacteria and archaea, producing methane-rich biogas and nutrient-rich digestate used as fertilizer. AD is prevalent in agriculture for manure management and energy production, primarily through single-stage systems, however two-stage systems which separate hydrolysis/acidogenesis from methanogenesis, show potential for faster, more stable treatment but lack proven industrial benefits (Adekunle & Okolie, 2015).

AD involves a complex reduction process under anoxic conditions comprising four biochemical steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Aslanzadeh, 2014). Hydrolysis is often rate-limiting for complex substrates due to toxic byproducts while methanogenesis limits easily biodegradable substrates (Adekunle & Okolie, 2015). The process relies on two microbial groups (acid-forming and methane-forming) whose balance is critical to avoid reactor instability and low methane yields.

Techniques like membrane separation and pH control enable phase separation based on differing growth kinetics (Adekunle & Okolie, 2015).

- **Hydrolysis**

Hydrolysis transforms insoluble organic compounds (e.g., lipids, polysaccharides) into soluble forms (e.g., monosaccharides, amino acids) using extracellular enzymes secreted by anaerobes like Bacteroides and Clostridia (Merlin Christy *et al*., 2014). The rate depends on substrate type, with cellulose degrading slower than proteins, and microbial specialization (saccharolytic or proteolytic) influences enzyme activity (Adekunle & Okolie, 2015).

- **Acidogenesis**

In acidogenesis, facultative and obligate anaerobes convert hydrolysis products into short-chain organic acids (e.g., acetic, butyric), alcohols, hydrogen, and carbon dioxide. Hydrogen concentration affects product formation with high partial pressure reducing yields of reduced compounds (Adekunle & Okolie, 2015).

- **Acetogenesis**

Acetogenesis involves anaerobic oxidation of acidogenesis products (e.g., volatile fatty acids, alcohols) into methanogenic substrates like acetate, hydrogen, and carbon dioxide. This phase requires collaboration with methanogens, who consume hydrogen to maintain low partial pressure, enabling inter-species hydrogen transfer (Aslanzadeh, 2014; Adekunle & Okolie, 2015).

- **Methanogenesis**

Methanogenesis, which is critical to the overall anaerobic digestion process efficiency is the slowest step and produces methane and carbon dioxide from acetogenesis intermediates using methanogenic bacteria under strict anaerobic conditions (Aslanzadeh, 2014).

BioFermentation

The fermentation bioprocess involves the biotransformation of a substrate rich in carbon and nitrogen to ethanol and other by-products like glycerol, lactic acid, acetic acid, enzymes, biopolymer, biofuels etc. based on which fermentative microorganism is used and the available mineral salts concentration (Bibra *et al*, 2023). Two groups of microbes (saccharolytic and ethanologenic) are important in bio-fermentation and they operate on the principle of co-metabolism, whereby, when saccharolytic microbes break down complex polymeric carbohydrates (starch, cellulose, hemicelluloses, etc.) to simpler utilisable forms, the ethanologenic converts them to ethanol (Gumisiriza *et al*.,2017). Notably, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are the only microbes naturally capable of producing ethanol close to theoretical maximum, with Saccharomyces cerevisiae predominant for current ethanol production based on starch and sugar feedstocks (Gumisiriza *et al*., 2017). Conversely, most organic waste contains cellulose, hemicellulose and lignin, and may require microbial consortia or genetically modified microbes to enhance breakdown and enable efficient cellulosic ethanol production (Gumisiriza *et al*., 2017). Bibra *et al*., (2023) have reported the fermentation of ethanol from organic food waste rich in carbohydrates (35.5-69%) and proteins (3.9-21.9%).

Enzymatic Hydrolysis

Enzymatic hydrolysis is a key method for treating food waste and producing bioethanol. This has advantages over chemical hydrolysis by eliminating the production of toxic byproducts and corrosion (Sakar *et al*., 2024). It breaks down polysaccharides in food waste into simple sugars (e.g., glucose, fructose, xylose) for fermentation, with glucoamylase being the dominant enzyme due to its ability to convert starches into glucose (Anwar Saeed *et al*., 2018). The efficiency of bioethanol production depends on the enzyme formulation tailored to the food

waste composition (Salimi *et al.*, 2019). Studies demonstrate high ethanol yields from various food wastes using enzymes like α-amylase, cellulase, and pectinase, with efficiencies ranging from 83–99.6% of theoretical yields (Ntaikou *et al.*, 2021).

Thermochemical Conversion Process

Thermochemical process involves decomposition of carbonaceous organic matter under high temperature to produce heat energy, fuel oil or gas and other value-added product such as charcoal. The main technological options under this category include incineration, pyrolysis, gasification and hydrothermal carbonization. Thermochemical process is useful for less dense wastes and low moisture content (Rajaeifar *et al.*, 2017). The most commonly available thermal technologies are discussed

Incineration

Incineration involves burning municipal solid waste (MSW) in oxygen at 800–1200°C for at least 2 seconds, producing heat, ash (bottom and fly), and flue gas (Alao *et al.*, 2022). This method requires minimal pre-treatment and converts heat into electricity or steam for district heating/industrial use through the steam Rankine cycle, with applications in power-only or combined heat and power (CHP) systems (Alao *et al.*, 2024; Patil *et al.*, 2024). Electricity is integrated into the grid, and heat enhances energy efficiency, though byproducts include ash and emissions (Patil *et al.*, 2024).

Pyrolysis

Pyrolysis thermally decomposes organic waste, biomass, and synthetic materials (e.g., plastics, rubber) in the absence of oxygen at 400–800°C, yielding biochar, bio-oil, and syngas (Patil *et al.*, 2024). Product quantity and quality depends on heating rate, temperature, residence time, feedstock, reactor type and catalysts (Alao *et al.*, 2024). Syngas supports electricity generation, bio-oil is used in biofuels/chemicals, and biochar serves as a fossil char substitute or soil enhancer (Al-Rumaihi *et al.*, 2022). Pyrolysis types (slow, fast, and flash) vary by retention time and temperature, with slow pyrolysis favoring biochar and fast/flash maximizing bio-oil (Alao *et al.*, 2024).

Gasification

Gasification converts biomass, municipal solid waste and solid fuels into syngas (containing methane, carbon monoxide, nitrogen, etc.) using gasifiers (20–500 kW) with gasifying media like heat, oxygen, and steam (Patil *et al.*, 2024). The process requires drying feedstock at 100°C and shredding for uniformity before gasification which occurs at 700–1000°C with limited oxygen to maximize fuel gas production, while minimizing condensable hydrocarbons and unreacted chars (Shahzad *et al.*, 2024). The choice of gasification technique depends on steam application and gasifying agents (Fang *et al.*, 2021). Syngas is used for electricity, biofuel production, or chemical synthesis, with tar and ash as byproducts (Patil *et al.*, 2024).

Hydrothermal Carbonization

Hydrothermal carbonization is an emerging technology for treating wet biomass, organic waste, and agricultural residues. Minimal pre-drying may be necessary. The temperature treatment (ranging from 180 ◦C to 250 ◦C) under high pressure yields hydrochar, which is a carbon rich material. Liquid by-products may also be produced, with the potential for soil amendment. The energy embedded in the hydrochar can be utilized for electricity generation or other applications contributing to the energy market. Moreover, the hydrochar can be applied as a soil conditioner, addressing the agricultural market's demand for sustainable soil improvement (Patil *et al.*, 2024).

Hydrothermal Liquefaction

Hydrothermal Liquefaction is a waste-to-energy process that converts feedstocks into high-energy bio-oil and other products (e.g., adhesives, resins, bio-polyols, polyurethane foams) under 4–25 MPa and 200–374°C (Sakar *et al*., 2024). The process involves hydrolysis of biomass into monomers, decarboxylation into smaller compounds and recombination into new compounds through condensation or polymerization (Gollakota *et al*., 2018). Catalysts enhance reaction kinetics and product quality; alkali catalysts (NaOH, KOH) reduce char formation and increase bio-oil yields, while acidic catalysts (H_2SO_4, H_3PO_4) lower temperature and reaction time (Sakar *et al*., 2024). Hydrothermal liquefaction (HTL), using water as a medium at 250–374°C and 4–25 MPa, is ideal for high-moisture feedstocks like algae, moisturized municipal solid waste, cattle manure, and sewage sludge, leveraging water's abundance and cost-effectiveness (Sakar *et al*., 2024).

Integrated Waste to Energy System

Pyrolysis–anaerobic digestion.

Anaerobic digestion generates digestate that can be dried and used in pyrolysis processes to generate biochar thereby improving energy sustainability and cost-efficiency (Begum *et al*., 2024). Integrating these processes enhances resource recovery from agricultural waste, boosting electricity yield by 42% compared to anaerobic digestion alone (Monlau *et al*., 2015). Biomass pretreatment improves bio-oil quality while biochar from digestate pyrolysis excels as a soil conditioner due to higher potassium, phosphorus, surface area, and water retention (Begum *et al*., 2024). Digestate derived bio-oils with fewer hydrocarbons, phenols and esters than raw food waste, resemble biodiesel and suit vehicle fuel needs. Improved biofuel recovery results from syngas biomethanation, which uses anaerobic microorganisms to convert CO_2, H_2, and CO into CH_4 (Yang *et al*., 2020; Begum *et al*., 2024). Combining liquid digestate and biochar improves soil quality making this approach sustainable for waste reduction and resources recovery in energy and agriculture (Begum *et al*., 2024).s

Hydrothermal liquefaction–anaerobic digestion

Anaerobic digestate management faces challenges like odor, environmental pollution, presence of pathogens and high costs or overuse when applied as liquid fertilizer (Kassem *et al*., 2020). Integrating hydrothermal liquefaction (HTL) with anaerobic digestion (AD) enhances energy recovery from organic waste by converting high-moisture anaerobic digestate into biocrude, a precursor to green transportation fuels (Kassem *et al*., 2020; Begum *et al*., 2024). HTL is well-suited for wet feedstocks like digestate, and post-HTL wastewater, retaining 40% organic matter and 80% nutrients serves as a sustainable resource for algae cultivation and further AD (Begum *et al*., 2024). This integrated HTL-AD approach maximizes resource recovery and material efficiency (Begum *et al*., 2024).

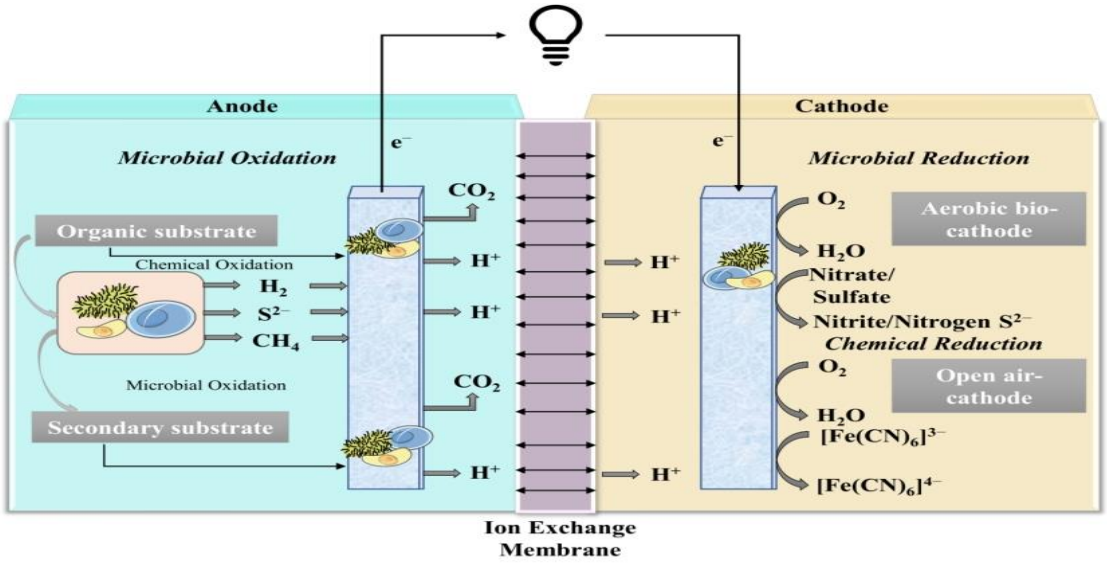
Hydrothermal Liquefaction–Fermentation

Hydrothermal liquefaction (HTL) converts high-moisture biomass into biocrude oils, producing a significant aqueous phase (HTL-AP) containing carbon, nitrogen, organics, inorganic components and heavy metal ions (Watson *et al*., 2020). Although typically considered waste, HTL-AP's rich chemical composition makes it a potential energy source. Anaerobic fermentation can transform HTL-AP into eco-friendly chemicals and fuels, but inhibitors may require pretreatment (e.g., extraction, partial oxidation, adsorption) to enhance biodegradability (Watson *et al*., 2020; Quispe-Arpasi *et al*., 2018). Alternatively, aerobic fermentation being faster and oxygen-dependent, is a viable method for producing value-added products from HTL-AP (Gu *et al*., 2019).

Innovatives Waste Conversion Techniquies

Bioenergy generation from microbial fuel cells

Microbial Fuel Cells (MFCs) utilize microbial metabolism to convert organic matter into bioenergy such as electricity, biohydrogen, and biogas (Slate *et al.*, 2019). In MFCs, microorganisms oxidize organic matter at the anode releasing electrons and protons which generate electric current as electrons flow to the cathode, reacting with an electron acceptor like oxygen to form water (Nawaz *et al*., 2020). Biohydrogen production in MFCs involves microbes like Clostridium spp. and Rhodobacter spp. using pathways such as dark fermentation and photofermentation, with strategies like metabolic pathway modification and optimal substrate selection to enhance yield (Ferreira *et al*., 2022; Pandya *et al*., 2024). Methane production is facilitated by electroactive methanogens, with electrochemical stimulation and optimized conditions improving output (Alves *et al*., 2022; Liu *et al*., 2023). Advances in electrode materials, such as carbon-based and novel options with increased surface area and biocompatibility, enhance electron transfer and power generation (Lorant *et al*., 2022). Synthetic biology improves electron transfer efficiency by engineering microbes with enhanced pathways or synthetic shuttles (Zhang *et al*., 2020). MFCs also treat wastewater by using organic pollutants as fuel, simultaneously generating electricity and reducing pollution, with ongoing pilot-scale applications (Malik *et al*., 2023).



Schematic representation of the working principle of MFCs. Adapted from Malik *et al*., 2023

Microbial Metabolism and Waste Valorization

Microorganisms degrade lignocellulosic biomass by secreting hydrolytic and lignin-degrading enzymes, breaking down the rigid cell wall structure and hydrolyzing biopolymers like hemicellulose and cellulose into monomeric sugars (Joshi *et al*., 2024). This process, involving cellulolytic and hemicellulolytic microbes, supports simultaneous fermentation to produce biofuels such as ethanol, furfural, and methane, alongside byproducts like acetate and organic acids (Reguera *et al*., 2015; Joshi *et al*., 2024). Notable microbes include bacteria (e.g., Bacillus sp., Cellulomonas sp.) and fungi (e.g., Aspergillus niger, Trichoderma reesei) known for biopolymer hydrolysis (Joshi *et al*., 2024). Advances in bioconversion involve synthetic control of microbial metabolic pathways to

enhance product yields, demonstrated in bioengineered *Escherichia coli* producing higher alcohols (e.g., isobutanol, 1-butanol) from glucose. This was achieved by amplifying 2-ketoacid decarboxylases and alcohol dehydrogenases, overexpressing genes like ilvIHCD and alsS from Bacillus subtilis and silencing genes for byproducts and pyruvate competition, yielding ~300 mmol/L (22 g/L) isobutanol under microaerobic conditions (Arancon *et al*., 2013).

Engineered Enzymes for Waste Degredation

Biorefineries enable sustainable conversion of organic waste into energy and high-value products, enhanced by metabolic engineering of microorganisms through gene deletion, overexpression, or pathway integration (Alibardi *et al*., 2020; Ben Tahar and Fickers, 2021). Saccharomyces cerevisiae, a key bioethanol producer, was engineered to metabolize xylose by overexpressing genes like Piromyces xylose isomerase and Pichia stipitis xylulose kinase, achieving high xylose consumption and ethanol titers (Ben Tahar and Fickers, 2021). Consolidated bioprocessing, that is combining hydrolysis and fermentation was achieved by expressing trifunctional cellulases from Clostridium cellulolyticum in S. cerevisiae EBY100, yielding 1.42 mg/L ethanol from cellulose. Biohydrogen production through dark fermentation was improved in E. coli by inactivating hycA and deleting genes for hydrogenases, lactate dehydrogenase and fumarate reductase achieving 2.11 mol/mol glucose, that is 90% of the theoretical maximum (Ben Tahar and Fickers, 2021). Succinic acid production was enhanced in Yarrowia lipolytica strain PGC202 by disrupting the succinate dehydrogenase gene (YALI0F11957g) and CoA-transferase gene (Ylach) enabling efficient production from fruit and vegetable waste at low pH with a 13-fold increase over the parental strain (Ben Tahar and Fickers, 2021).

Circular Economy and Waste Upcycling

Global waste generation reaching 2.12 billion tons annually poses significant environmental challenges with most waste sent to landfills emitting pollutants (Kish, 2016). A circular economy aims to mitigate climate change and promote sustainability by converting waste into high-value products through mechanical, thermochemical, and biochemical processes, prioritizing chemical recycling over simple reuse (Kish, 2016; Sung, 2023). Traditional methods like incineration are inefficient producing pollutants such as mercury, dioxins and carbon dioxide, while anaerobic digestion is limited to biodegradable waste leaving digestate with odor, pathogens, and heavy metals that risk environmental contamination (Kassem *et al*., 2020; Kish, 2016). Emerging (integrated) waste-to-energy technologies like hydrothermal liquefaction–anaerobic digestion and pyrolysis–anaerobic digestion enables the conversion of both biodegradable and non-biodegradable waste into clean energy products like hydrogen, synthetic fuels, and “green” chemicals, reducing landfill use and fossil fuel dependency (Kish, 2016; Begum *et al*., 2024). These technologies require efficient processes and reliable cleaning systems to prevent pollution, with product outcomes varying by feedstock and processing conditions (Kish, 2016).

Challenges and Future Directions

Despite its promise, waste valorization faces significant hurdles such as;

Technological Limitation: Many processes remain energy-intensive or economically unviable at scale. For example, enzymatic recycling of plastics is still slower and costlier than mechanical method.

Waste Heterogeneity: Mixed or contaminated waste streams complicate processing; necessitating advanced sorting and pretreatment technologies.

Policy and Behavioral Gaps: Inconsistent regulations and consumer resistance to recycled products hinder market adoption.

Conclusion

Waste valorization represents a transformative opportunity for biochemists to address the global waste crisis while advancing sustainable innovation. By leveraging cutting-edge biochemical techniques, such as anaerobic digestion, microbial fermentation, enzymatic catalysis, and synthetic biology, this field unlocks the potential of waste materials to produce high-value products like biofuels, biodiesel, bioplastics, biopolymers and bioactive compounds. The investigation of new frontlines in waste valorization not only mitigates environmental degradation and resource scarcity but also fosters a circular bioeconomy, aligning with global sustainability goals. This study underscores the critical role of biochemists in driving technological breakthroughs, overcoming scalability challenges, and creating economic opportunities through waste-to-resource conversion. Ultimately, waste valorization paves the way for a resilient, resource-efficient future, where waste is no longer a burden but a valuable asset for scientific, environmental, and societal progress.

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(JNASR) Journal of Natural and Applied Sciences Research
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Evaluating Police Awareness and Utilization of Forensic Science in Criminal Investigations: Evidence from the Edo State Police Command, Nigeria.

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Abstract

Forensics can be used interchangeably with forensic science, it is the application of scientific expertise for the resolution of legal disputes, both criminal and civil. The main aim of forensic science is to gather intelligence to enable the judge to credible and logical decisions in the court by means of scientific approach through evaluation of evidence for the administration of justice, and country around the world now considers forensic methodology as the gold standard for criminal investigation. Therefore, the present study assessed the awareness level on the significance of forensics in criminal investigation in Nigeria: a case study of Edo State Police command. The questionnaire comprised of two main categories: Socio-demographic characteristics, and Information on the relevance of forensics in criminal investigation in Nigeria. For the analysis of data collected; the statistical tool used was Statistical Package for Social Sciences, version 22 for windows. The result of the present study revealed that the participants were distributed social demographically as follows; there was an observable higher number of male participants (68%) relative to the female participants (32%), As per age distribution, a larger population of the participants were found to be >40 years of age with 55%, and it was observed that age between 35-39 years ranked the least with 15%. On educational level, the result of the present study revealed that majority of the participants possesses a bachelor's degree as the highest level of educational qualification with 75% from a pool of 100% of participants. The present study further examined responses on the relevance of forensics in criminal investigation, and the result revealed an inadequate level of awareness on the relevance of forensics in criminal investigation. Therefore, the study recommends that the Nigerian Police Force and the Judiciary should collaboration with universities running programs on forensics for trainings.

INTRODUCTION

Forensic science refers to the application of natural, physical, and social sciences to matters of the law. Most forensic scientists hold that investigation begins at the scene, regardless of their associated field (Marasa & Miranda, 2014). The proper investigation, collection, and preservation of evidence are essential for fact-finding and for ensuring proper evaluation and interpretation of the evidence, whether the evidence is bloodstains, human remains, hard drives, ledgers, and files or medical records (Marasa & Miranda 2014).

Forensic science is a multidisciplinary field drawing principally from chemistry and biology, but also psychology and social sciences. Its relevance in the criminal justice system cannot be overemphasized, as the need to have a crime-free, peaceful, habitable and developed society continually subsists (Ladapo, 2011). The activities of the forensic scientists include; deoxyribonucleic acid (DNA) matching, hair analysis, serology test, fingerprint analysis, blood spatter analysis, crime scene investigation, etc. The main aim of forensic science is to gather intelligence to enable the judge to credible and logical decisions in court by means of scientific approach through evaluation of evidence for the administration of justice (Cardinetti, &Cammarota, 2005).

In a country like Nigeria where the crime rate is on the surge and the government seems helpless because of the sophistication in terms of how such crimes are perpetrated. Experts have linked many cases of unsolved crimes that dot the Nigeria criminal justice system to the absence of forensic evidence. This forensic gap has rendered justice quite protracted and ineffective (Ngboawaji, 2012). In Nigeria, serious crimes such as murder continue to remain unsolved by the criminal justice system due to the gap in forensic science. Nigeria Police Force and other security agencies in Nigeria rely heavily on eye witness testimonies, circumstantial evidence, and confessions where others fail. A combination of two or all of them is a thorough and detailed investigation done as far as they are concerned. Whereas, the most important source of evidence; forensic evidence which is more reliable, authentic, concrete and productive are underutilized (Alisigwe and Oluwafemi, 2019). This is a serious dent on the forensic investigative ability of the Nigerian police and other such security agencies. The identification of murder suspects is a critical element in forensic investigation (Alisigwe and Oluwafemi, 2019; Alemika & Chukwuma, 2006).

The role of forensic science services in the administration of justice starts at the crime scene with the recognition and recovery of physical evidence. It proceeds with its analysis and the evaluation of the results in a laboratory, and the presentation of the findings to judges, prosecutors, lawyers and others in need of the factual information. From the first responders to the end-users of the information, all personnel involved should have an adequate understanding of the forensic process, the scientific disciplines and the specialized services provided by forensic laboratories (Alisigwe and Oluwafemi, 2019). Forensic science provides multiple options that would help in tracking and apprehending perpetrators of such dastardly acts by agents of the criminal justice system. The role of forensic science is changing from a supporting role to the playmaker in many types of investigation, providing quick, objective and reliable information on crime and suspects (Tjin-A-Tsoi, 2014). Therefore, the present study assessed the awareness level on the significance of forensics in criminal investigation in Nigeria: a case study of Edo State Police command.

MATERIALS AND METHODS

Research Design

The design used in this study is the survey research design. The type of survey design is section survey research design; it collects data immediately from respondent. This design is considered appropriate because all the variables of the study will be observed at a point in time and thereby relatively reducing the cost of the investigation.

Population and Sampling of the Study

The population of this study was the Edo State Police Command. The sample size of this study is a total number of one hundred (100) police officers in Edo State Command drawn from our population for empirical investigation. The sample size is what is considered adequate in order to avoid sampling error in our results. This is on account that we cannot statistically derive our sample size since the finite size of our population is not certain. The sampling method employed in this study is convenience sampling techniques.

Sources of Data

The sources of data employed in this study work include the primary data and secondary sources of data. Primary data sourced from questionnaire was mainly used in this study. Secondary source consisted of textbooks, journals, libraries, magazines and articles.

Validity and Reliability Test

This research study has been validated by my supervisor by reading my work and making corrections and this correction have been implemented. And hence has approved the validation of the study. Data from the primary sources are very reliable. It has been tested over and over again and the same results were obtained which means the information was very reliable.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Version 22 and Microsoft Excel.

RESULTS

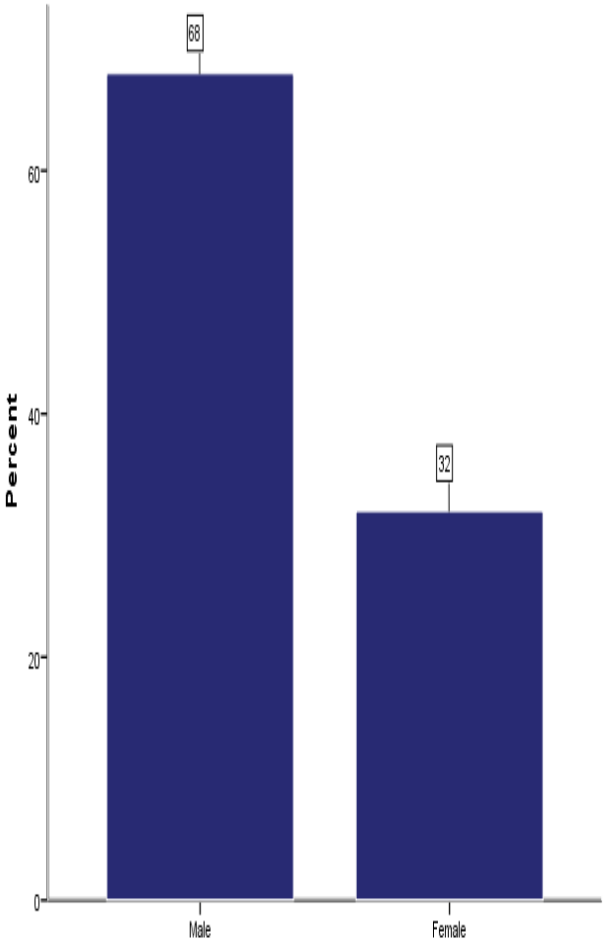

Figure 1: Showing Gender Distribution

The result of the present study on gender distribution, there was an observable higher number of male participants (68%) relative to the female participants (32%).

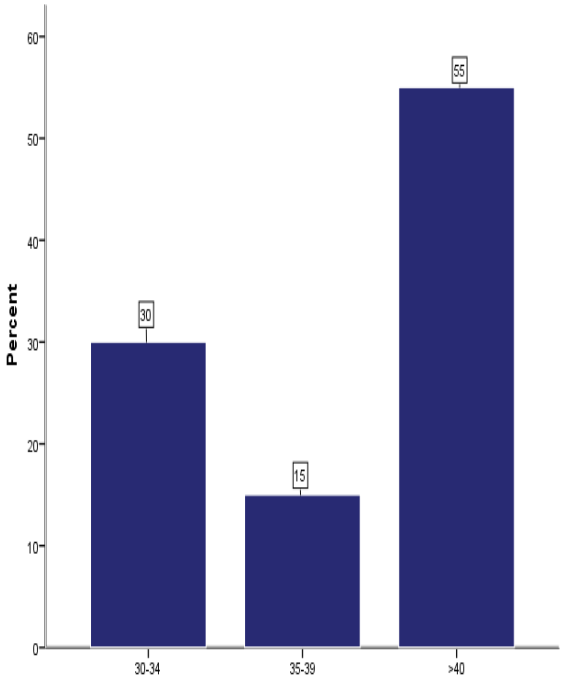

Figure 2: Showing Age Distribution.

The result presented in figure 2 revealed that the >40 years was the predominant age distribution of the participants and the age between 35-39 was observed to be the least.

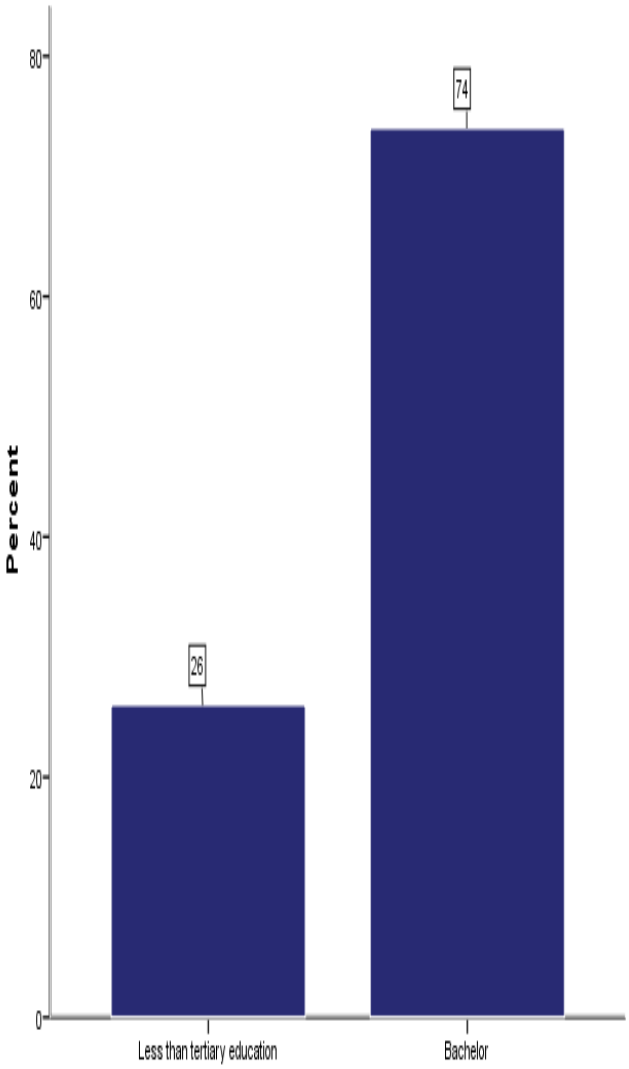

Figure 3: Showing Educational Level

From the total population of one hundred participants, it was observed that a large proportion of the participants had a bachelor degree as the highest academic qualification (77%) and a few others 26% were found to have less than a tertiary educational qualification.

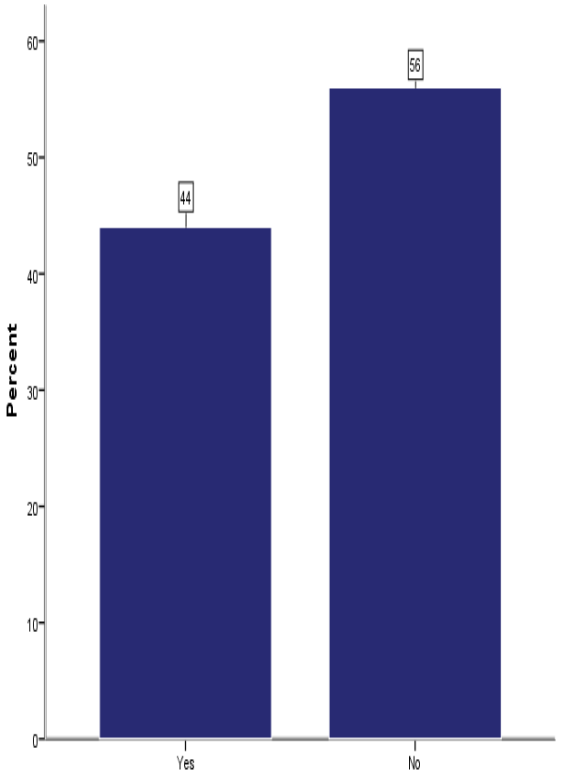

Figure 4: Showing responses on knowledge about forensic sciences

The result on the knowledge of forensics science as demonstrated in figure 4 revealed that, a greater population (56%) of the participants from a pool of 100% had no knowledge about forensics, and only 46% of the population demonstrated adequate knowledge of forensics.

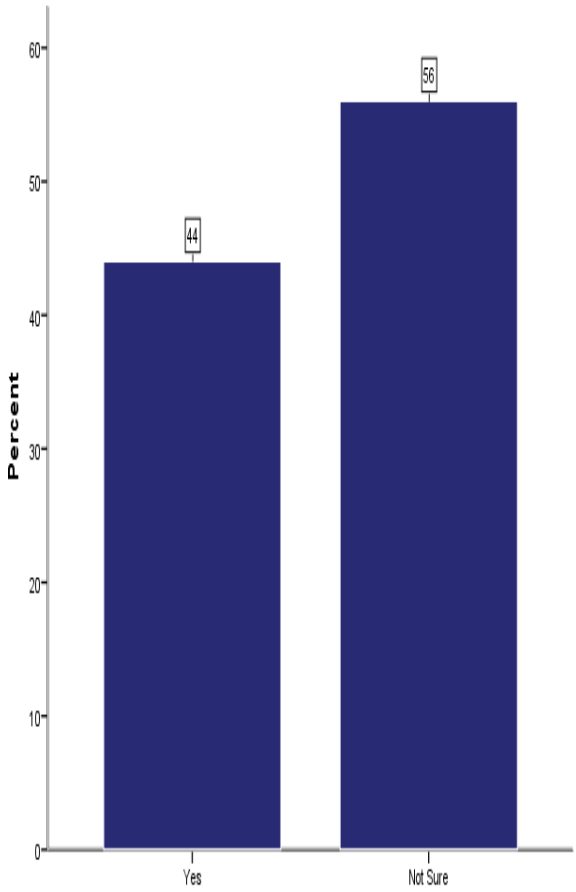

Figure 5: Showing responses on can forensics methodology be used for criminal investigation in Nigeria.

The result on can forensics methodology be used for criminal investigation in Nigeria as shown in figure 5 revealed that, a greater population (56%) of the participants from a pool of 100% were not sure, and only 44% of the population demonstrated adequate knowledge of forensics.

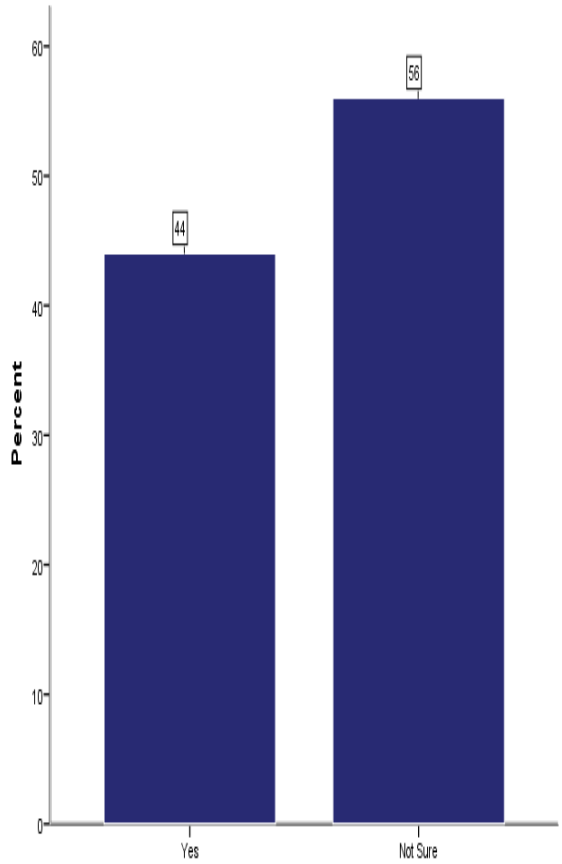

Figure 6: Showing responses on does forensics provide irrefutable evidence for the administration of justice.

The result shown in figure 6 on does forensics provide irrefutable evidence for the administration of justice, a significant proportion of the participants revealed were not sure and a minimal proportion from the pool of total population 44% agreed.

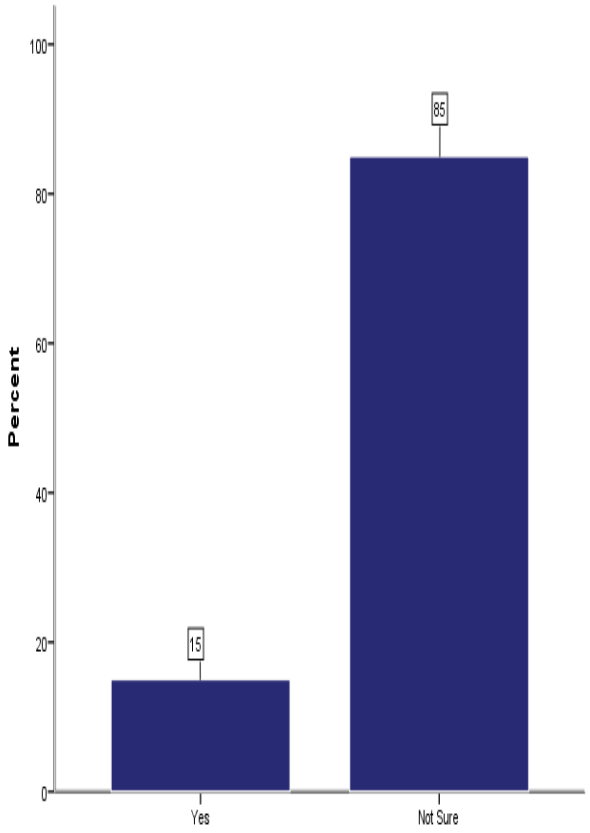

Figure 7: Showing responses on does Nigeria courts accepts forensic evidence

The result as shown in figure 7 on does Nigeria courts accepts forensic evidence, revealed that majority of the participants were not sure and only a little fraction of the population 15% agreed that Nigeria courts accept forensic evidence.

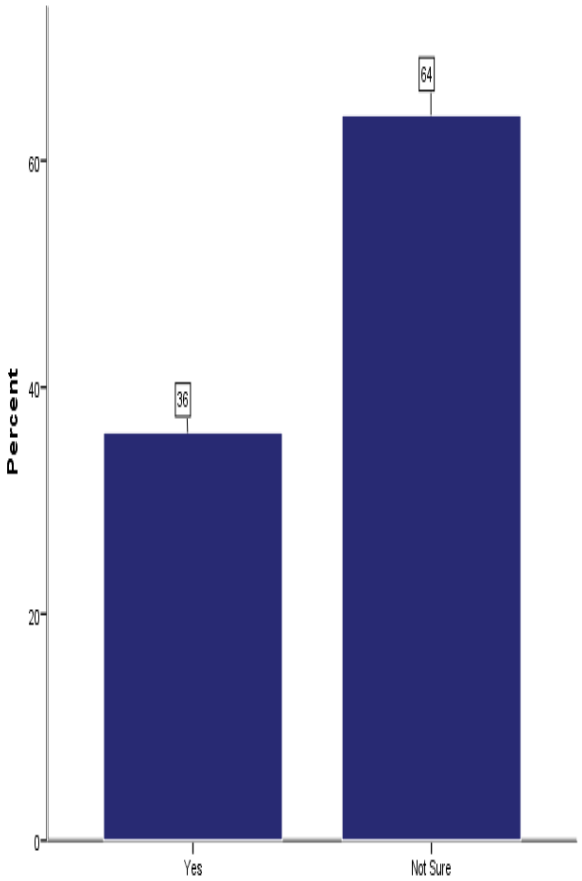

Figure 8: Showing responses on would forensics contribute to the reduction of crimes in Nigeria

Figure 8 showed that from the total pool of the study population, a low proportion of the participants 36% agreed that forensics contributes to the reduction of crimes in Nigeria.

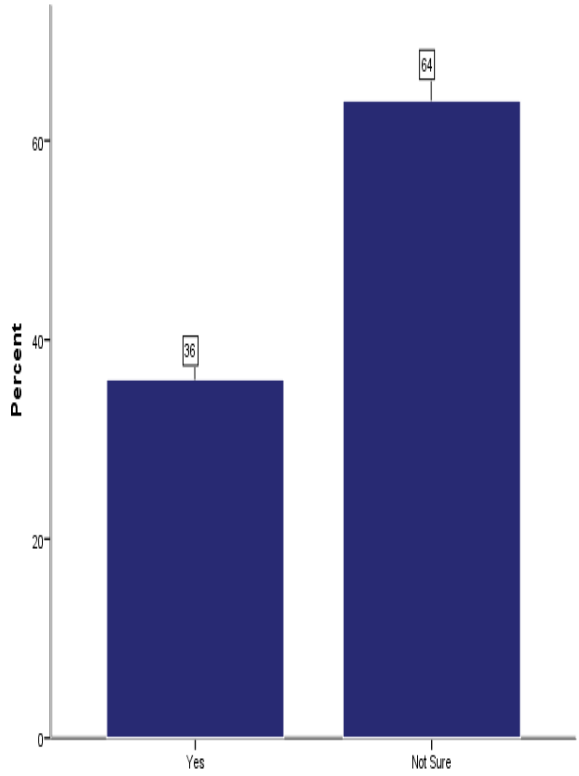

Figure 9: Showing responses on can forensics be used to tackle the upsurge of crime in Nigeria.

Figure 9 revealed that majority of the participant 64% were not sure if forensics can be used in tackling the upsurge of crime in Nigeria. Conversely, 36% agreed to the response forensics can be used to tackle the upsurge of crime in Nigeria.

Discussion

Forensic science is one of the significant elements of criminal justice system. It basically deals with the exploration of scientific and physical clues gathered from the crime scene. The need for the application of this Science in criminal investigation arose because our society has been undergoing drastic societal changes at a very rapid pace (Narejo and Avais, 2012). We saw a growing shift from a rural society to an urban one which left the old technique of criminal investigation obsolete. Consequently, there is a growing need of forensic Sciences to identify the different types of crimes and

criminals and the techniques which are used by the offenders. It is important because when scientific techniques and methods are used there is not much scope for any biases or injustices (Narejo and Avais, 2012; Faiza et al., 2020). Hence, the present study examined the awareness level on the significance of forensics in criminal investigation in Nigeria: a case study of Edo State police command

The result of the present study on social demographic characteristics as shown in figure 1-4 revealed the following. For gender; from the study population of 100 participants, there was an observable higher number of male participants (68%) relative to the female participants (32%). Our result on gender distribution corroborates similar report of (Nwawuba and Akpata, 2020; Oguntunde, 2012). As per age distribution, a larger population of the participants were found to be >40 years of age with 55%, and it was observed that age between 35-39 years ranked the least with 15%. On educational level, the result of the present study revealed that majority of the participants possesses a bachelor's degree as the highest level of educational qualification with 75% from a pool of 100% of participants, while 26% of the participants was found to posses less than tertiary education as the highest qualification. The inclusion criteria of participants of Law enforcement, is as a result of their involvement in criminal investigation. of course, the criminal justice system (CJS) is an essential part of any civilized nation to ensure justice, fairness, the practice of the rule of law and the institutionalization of a democratic system (Olonisakin et al., 2017). A criminal justice system is a system made up of different agents charged with the responsibilities of investigating and prosecuting criminal cases and the system envisages the law enforcement, and judiciary as its components (Olonisakin et al., 2017; Alemika, 2014). Correspondingly, body of evidence has revealed that the law enforcement and the judiciary collaborate with forensic scientist in criminal investigation (Nwawuba and Akpata, 2020; Nwawuba et al., 2021), hence the study focused on examining the level of awareness on the relevance of forensics in criminal investigation among the law enforcement agencies.

The present study further examined responses on the relevance of forensics in criminal investigation. the result on have you heard about forensics as demonstrated in figure 4 revealed that, a greater population (56%) of the participants from a pool of 100% had no knowledge about forensics, and only 46% of the population demonstrated adequate knowledge of forensics. The same trend was also observed on can forensic methodology be used for criminal investigation in Nigeria (figure 5) as well the responses on does forensics provide irrefutable evidence for the administration of justice (figure 6) with 56% (Not Sure) and 46% (Yes). Forensics can be used interchangeably with forensic science, it is the application of scientific expertise for the resolution of legal disputes, both criminal and civil (Nwawuba et al., 2020). The main aim of forensic science is to gather intelligence to enable the judge to credible and logical decisions in the court by means of scientific approach through evaluation of evidence for the administration of justice, and country around the world now considers forensic methodology as the gold standard for criminal investigation (Cardinetti, &Cammarota, 2005; Nwawuba et al., 2021; Machado and Silva, 2019). Additionally, forensics facilitates criminal investigation in all varied facets and plays the pivotal role in keeping law and order in a society, and in today's world the use of forensic evidence and the application of forensic sciences form an integral part of the criminal investigation system and prosecutions (Gowsia and Sheeba, 2018; Faiza et al., 2020).

As per the responses on do Nigeria courts accepts forensic evidence, the result as demonstrated in figure 7 revealed that a larger proportion of the participants were not sure (85%) while only 15% (Yes) demonstrated adequate knowledge on the position of forensic evidence in the Nigeria justice system. This result agrees to the assertion that there is dearth in knowledge on forensics as well as the laws that allows for acceptance of forensic evidence in the Nigerian courts (Alisigwe and Oluwafemi, 2019; Obafunwa, 2018). In practice, the Nigerian courts are placed in a position to accept evidence relating to the use of scientific evidence, and the person empowered to present such evidence is regarded as an expert witness as contained in Evidence Act 2011, s 68 (Kehinde, 2014; Nwawuba et al., 2021).

Finally, responses on would forensics contribute to the reduction of crimes in Nigeria, and can forensics be used to tackle the upsurge of crime in Nigeria as shown in figure 8 & 9 followed similar trend with 64% of the participants reporting (Not Sure) and only 36% from the pool of 100% reported with a (Yes). The relevance of forensics in the criminal justice system cannot be overemphasized, as the need to have a crime-free, peaceful, habitable and developed society continually subsists (Ladapo, 2011). Regardless of the severity of a criminal case, it has been proven that forensics continues to be a reliable workhorse for criminal investigation, and the result of forensic investigation can make the difference between the acquittal and conviction in the court of law (Gowsia and Sheeba, 2018). It is unbelievable that with the advancement of technologies for forensics in crime investigation, the Nigerian Police Force still to a great extent relies on traditional investigative techniques for criminal investigation (Alisigwe and Oluwafemi, 2019). The present-day Nigeria is faced with security challenges and it has been revealed that criminals have become more sophisticated in their operations, and the peculiarity of the various forms of crime committed in Nigeria tends to involve or leave behind biological evidence at the scene of a crime. These biological shreds of evidence are a key sample for DNA profiling and subsequent storage in a forensic DNA database (Nwawuba et al., 2020). Hence, a switch to a modern approach of criminal investigation "forensics" has become very necessary to detect, apprehend, and enhance the administration of justice.

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(JNASR) Journal of Natural and Applied Sciences Research
Izevbuwa *et al.*, (2025). 1(1): 89-102. Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Antimicrobial Resistance in Pregnancy: Prevalence and Characterization of ESBL-Producing Uropathogens in a Nigerian Tertiary Hospital

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Abstract

Extended-spectrum beta-lactamases (ESBLs) are increasingly contributing to antibiotic resistance, particularly in urinary tract infections (UTIs) among pregnant women. This study aimed to determine the prevalence of ESBL-producing bacterial strains and assess their antibiotic resistance profiles in urine samples collected from pregnant women at General Hospital Ilorin. Antimicrobial resistance (AMR) remains a critical global health issue, compounded by the rise of multidrug-resistant (MDR) organisms such as ESBL producers. These pathogens pose significant challenges in infection control, especially in pregnant women who are more susceptible to UTIs. A six-month cross-sectional study was conducted in the Department of Medical Laboratory Science at General Hospital Ilorin. A total of 250 urine samples were aseptically collected from pregnant women aged 15–40 years who were clinically suspected of having UTIs. The samples were screened for ESBL-producing bacteria using selective media, and antibiotic susceptibility testing was performed using standardized protocols. Out of the 250 samples analyzed, 95 (38%) demonstrated significant bacterial growth. The predominant uropathogens were *Escherichia coli* (14%) and *Klebsiella* species (24%), with *Klebsiella pneumoniae* and *Klebsiella oxytoca* each constituting 12% of the isolates. The highest infection rates were observed among women aged 21–30 years. ESBL-producing isolates exhibited resistance to commonly used antibiotics including gentamicin, levofloxacin, ceftazidime, and ciprofloxacin, while maintaining susceptibility to imipenem and piperacillin. The findings of this study highlight a worrisome prevalence of ESBL-producing bacteria and underscore the growing challenge of antibiotic resistance in pregnant women. A strong correlation was observed between ESBL production and multidrug resistance, emphasizing the urgent need for continuous surveillance, proper antibiotic stewardship, and the development of effective treatment strategies tailored for this vulnerable population.

Introduction

The history of β-lactam antibiotics began in 1928, when Alexander Fleming observed that the *Penicillium* mold secreted a substance with antibacterial properties (Sebastian *et al*., 2019). Although Fleming recognized the importance of his discovery, it was not immediately pursued or validated, particularly due to the limited focus on its potential during World War II (Kardos and Demain, 2013). There was initially considerable debate regarding the chemical structure of penicillin discovered by Fleming. This uncertainty was resolved in 1945 through the work of Dorothy Hodgkin, who determined that penicillin contains a four-membered ring structure known as the β-lactam ring, or 2-azetidinone (Sebastian *et al*., 2019). The β-lactam structure was viewed with suspicion since it was thought that its ring strain would remove the usual amide resonance, making it extremely reactive with nucleophiles. This notion appeared to be supported by early synthesis difficulties in turning β-amino acids into β-lactams. But by applying carbodiimide activation, Sheehan eventually broke through this synthetic barrier and famously called the β-lactam the "enchanted ring." However, simple β-lactams do not demonstrate exceptional reactivity in acyl transfer reactions with nucleophiles, according to later experimental studies (Fisher *et al*., 2016). Despite this, the β-lactams used as antibiotics are more complex than the basic β-lactam ring. Their antibacterial activity depends on carefully tuned reactivity towards nucleophiles and the presence of a nearby negative charge for biological recognition (Pratt *et al*., 2010). In penicillins, cephalosporins, and carbapenems, this activation is achieved by fusing a second ring to the β-lactam, and a carboxylate group provides the required negative charge. In contrast, monobactams and monosulfactams integrate both the activating features and negative charge through specific functional groups attached to the nitrogen atom of the β-lactam ring.

Methodology

A cross-sectional descriptive study was conducted at the Department of Medical Laboratory Science, General Hospital Ilorin, Kwara State, Nigeria. The research spanned a period of six months and focused on evaluating the prevalence of Extended Spectrum Beta-Lactamase (ESBL)-producing bacteria in pregnant women with symptoms suggestive of urinary tract infections (UTIs). This study included 250 pregnant women aged between 15–40 years, who were suspected to have UTIs based on clinical signs and symptoms. A purposive sampling technique was employed to select participants. Each subject provided a single midstream urine sample for microbiological evaluation. Clean-catch midstream urine samples were collected aseptically using sterile, wide-mouth, leak-proof universal containers. Participants were educated on proper sample collection procedures to avoid contamination. Collected samples were promptly labeled and transported to the laboratory within one hour under cold chain conditions for immediate processing. Urine samples were subjected to microbiological analysis immediately upon arrival at the laboratory. Each specimen was thoroughly mixed and inoculated using a standard calibrated platinum wire loop delivering 0.001 mL of urine. Inoculation was performed on two types of culture media which were Cysteine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar. After inoculation, the agar plates were incubated in an aerobic environment at 37°C for 18 to 24 hours. Following incubation, plates were examined for colony morphology, color, hemolysis, and lactose fermentation status. Colony counts were estimated by multiplying the number of colonies by the loop factor (1000), and results were expressed in colony-forming units per milliliter

(CFU/mL). A bacterial count of $\geq 10^5$ CFU/mL was interpreted as significant bacteriuria, consistent with clinical infection criteria, especially in asymptomatic or mildly symptomatic individuals. Mixed growths or counts below the threshold were considered contamination unless clinically justified and supported by microscopy and symptomatology. Isolated bacterial colonies were first subjected to Gram staining, a differential staining technique used to classify bacteria into Gram-positive or Gram-negative groups based on the composition of their cell walls. This initial step provides essential information regarding the bacterial morphology and structural characteristics. Following Gram staining, a series of biochemical tests were performed to further identify the species of the isolated bacteria. Presumptive ESBL production was screened using MacConkey agar supplemented with cefotaxime and ceftazidime (2 µg/mL each). Isolates that showed reduced susceptibility were subjected to the combined disc synergy test (CDST) using discs of ceftazidime (30 µg) and cefotaxime (30 µg) alone and in combination with clavulanic acid (10 µg). A ≥5 mm increase in zone diameter in the presence of clavulanic acid was interpreted as ESBL positive. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Plates were incubated at 37°C for 18–24 hours, and the zones of inhibition were measured and interpreted according to CLSI breakpoints. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial categories. Plates were incubated at 37°C for 18 to 24 hours, after which the zones of inhibition around antibiotics were measured and interpreted using CLSI breakpoints to determine susceptibility. Multidrug resistance was defined as resistance to at least one antibiotic in three or more different antimicrobial categories. To identify the bacteria, several biochemical tests were performed. The urease test involved using urea broth or slants, where a color change to pink indicated positive urease activity. The indole test assessed the bacteria's ability to metabolize tryptophan, with a red ring forming after adding Kovac's reagent to indicate a positive result. The citrate utilization test was done using Simmons' citrate agar, where a color change from green to blue showed the bacteria could utilize citrate. The triple sugar iron test evaluated the fermentation of glucose, lactose, and sucrose, as well as hydrogen sulfide production, by observing changes in color and gas formation. The motility test used motility agar, where the presence of diffused growth away from the stab line confirmed motility. Together, these biochemical tests helped confirm the identity of ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* isolates.

Summary table of current options to treat infections due to ESBL-producing Enterobacteriaceae in different groups of patients

Group[1]	Characteristics of infection in each group	Options for treatment (dosing with normal renal function)
1	Severe infections; or Infections with a high-risk source; or/and Severely-immunocompromised patients	Imipenem (500 mg/6 h) Meropenem (1 g/8 h)
2	Non-severe infections from intermediate-risk source	Ertapenem (1 g/day) Piperacillin-tazobactam (4.5 g/6–8 hr in extended infusion) Imipenem (500 mg/8 hr) Meropenem (1 g/8 hr)
3	Non-severe urinary tract infection	Ertapenem (1 g/day) Piperacillin-tazobactam (4.5 g/6–8 hr in extended infusion) Amoxicillin-clavulanic acid (intravenous: 2.2 g/8 hr; oral for UTI: 1.250 g/8 hr) Aminoglycosides (amikacin: 15–20 mg/kg/day; gentamicin or tobramycin: 5–7 mg/kg/day) Plazomycin (15 mg/kg/day) Cephamycins (flomoxef: 1 g/8 hr; cefmetazole: 1 g/8 hr) Imipenem (500 mg/8 hr) Meropenem (1 g/8 hr) Fosfomycin iv (4 g/6 hr) Trometh amol-oral for non-bacteraemic UTIs (3 g/48 hr) Ciprofloxacin-oral for non-bacteraemic UTIs (400 mg/8–12 hr)

Fig 1: Current Options for treatment of infections due to ESBL-producing Enterobacteriaceae (Gutierrez-Gutierrez and Rodriquez-Bano, 2019).

RESULT

Table 1: A total of 250 urine samples were collected from pregnant women for this study. Out of these, 95 samples (38.0%) showed significant bacteriuria upon culture. Among the positive cultures, Escherichia coli was isolated from 35 cases, while Klebsiella species (including *Klebsiella pneumoniae* and *Klebsiella oxytoca*) were identified in 60 cases. The distribution of isolates indicated that Klebsiella species accounted for nearly half of all bacterial isolates, with *Klebsiella pneumoniae* and *Klebsiella oxytoca* each contributing 30 isolates (12.0%), making up a combined 24.0% of the total specimens analyzed.

Table 1: Prevalence of uropathogens isolated from the specimens

Organisms	Number of specimen	Percentage (%) Occurrence
Escherichia coli	*35*.00	14.00
Klebsiella pneumonia	*30*.00	12.00
Klebsiella oxytoca	30.00	12.00
Total	95.00	38.00

Table 2: In the 15–20 age group, bacterial presence is minimal, with only *Klebsiella pneumoniae* and *Escherichia coli* detected each in 5 cases, totaling 10. In contrast, the 21–25 age group shows a lower overall case count of 5 but a more varied bacterial profile like the *Klebsiella oxytoca* and *Escherichia coli* appear in 10 cases each, while *Klebsiella pneumoniae* is found in 5 cases. The 26–30 age group also reports 25 cases, with *Escherichia coli* being more prevalent at 15 cases and *Klebsiella pneumoniae* at 10. no *Klebsiella oxytoca* cases are observed in this group. For individuals aged 31–35, the distribution is relatively even, with `10 cases of *Klebsiella oxytoca*, and 5 cases each of *Klebsiella pneumoniae* and *Escherichia coli*, amounting to 20 cases. Lastly, the 36–40 age group records a slight decline in bacterial cases, showing 10 instances of *Klebsiella oxytoca* and 5 of *Klebsiella pneumoniae*, while *Escherichia coli* is absent, making a total of 15 cases.

Table 2: Prevalence of bacteria species in relation to age

Age group	Klebsiella *oxytoca*	*Klebsiella pneumoniae*	*Escherichia coli*	Total
15-20	0	5	5	10
21-25	5	5	10	25
26-30	0	10	15	25
31-35	10	5	5	20
36-40	10	5	0	15
Total	**25**	30	35	95

Table 3: Analysis of specimen growth across age groups revealed that individuals aged 21–25 and 26–30 exhibited the highest levels of bacterial growth, each with 25 cases, accounting for 10.00% respectively. In contrast, the lowest growth was observed in the 15–20 age group, with only 10 cases

(4.00%), as shown in Table 3. Furthermore, the distribution of *Klebsiella* species and *Escherichia coli* across age groups indicated that patients aged 21–25 and 26–30 years had the highest prevalence, each contributing 25 cases (10.00%) to the total.

Table 3: Prevalence of bacteria species in relation to age giving in percentage

Age group(years)	Number examined (%)	Number of significant growth (%)
15-20	45(18)	10(4)
21-25	50(20)	25(10)
26-30	55(22)	25(10)
31-35	60(24)	20(8)
36-40	40(16)	15(6)
Total	250(100)	95(38)

Table 4: Out of the 95 bacterial isolates analyzed, *Escherichia coli* demonstrated alarming resistance trends. Every isolate (100%) was resistant to Augmentin, Ciprofloxacin, Ceftazidime, and Imipenem. This widespread resistance to vital antibiotics including penicillins and cephalosporins strongly suggests the presence of extended-spectrum beta-lactamase (ESBL) enzymes, which deactivate these drugs by breaking down their beta-lactam structures. Supporting this, 85.7% of *E. coli* isolates also showed resistance to piperacillin-tazobactam. However, 14.3% remained sensitive to piperacillin-tazobactam, and notably, all isolates (100%) were susceptible to both nitrofurantoin and amikacin. *Klebsiella pneumoniae* displayed a similarly high resistance pattern, with 100% resistance to ceftazidime, Augmentin, Imipenem, and Ciprofloxacin.

Table 4: Antibiotic susceptibility pattern of bacternuria Isolates

Bacterial IsolatesPattern	AST	AUG	LEV	CAZ	IMI	PTZ	NI	CRO	AK	GM

Eschericia coli S		5(14.3)	35(100)		35(100)		-	
(*n=35*)	**I**	25(71.4)					-	
	R	35(100)	10(28.5)	35(100)	35(100)	30(85.7)	35(100)	-
Klebsiella	**S**				30(100)	-	30(100)	20(66.6)
pnemoniae (n=30)	**I**		5(16.6)					2(28.6)
	R	**30**(100)	25(83.3)	30(100)	30(100)	-	30(100)	
Klebsiella	**S**				30(100)	-	20(66.6)	
Oxytoca(n=6)	**I**		**15**(50)			-	10(28.6)	
	R	**30**(100)	15(50)	30(100)	30(100)	-	30(100)	30(100)
Total (*n=95*)	**S**				65(68.4)	7(100)	85(89.4)	20(33.3)
	I		45(47.3)				10(10.5)	10(16.6)
	R	95(100)	50(52.6)	95(100)	95(100) 3	0(31.5)	95(100)	30(50)

AUG: augumentin, LEV: levofloxacin, CAZ: ceftazdime, IMI:imipenem PTZ: piperacillin-tazobactam,NI: nitofurantoin, AK:amikacin, GM: gentamcin,CRO:ciprofloxacin

The observed resistance pattern, including 83.3% resistance to Levofloxacin, is consistent with the behavior of ESBL-producing organisms, which are capable of hydrolyzing beta-lactam antibiotics and rendering them ineffective. All *Klebsiella pneumoniae* isolates were fully sensitive to Amikacin and Piperacillin-Tazobactam, and 66.6% remained susceptible to Gentamicin, suggesting these antibiotics still offer some therapeutic potential despite widespread resistance. *Klebsiella oxytoca* isolates also showed complete resistance (100%) to Augmentin, Ciprofloxacin, Imipenem, Ceftazidime, and Gentamicin, and demonstrated 50% resistance to Levofloxacin. These resistance trends further point to ESBL activity, as the bacteria's ability to neutralize beta-lactam antibiotics supports the presence of such enzymes. However, all *K. oxytoca* isolates were fully susceptible to Piperacillin-Tazobactam, and 66.6% were responsive to Amikacin. Across all 95 isolates, there was universal resistance to Augmentin, Ciprofloxacin, Imipenem, and Ceftazidime. Levofloxacin resistance was recorded at 52.6%, while Piperacillin-Tazobactam showed 31.5% resistance. Among *Klebsiella* species, Gentamicin resistance reached 50%. On the other hand, complete sensitivity to Nitrofurantoin was noted (excluding *Klebsiella* spp.), Amikacin had an 89.4% sensitivity rate, and Piperacillin-Tazobactam showed 68.4% effectiveness. Gentamicin had the lowest susceptibility overall, at just 33.3%.

DISCUSSION

This study focused on the frequency and distribution of significant bacteriuria in urine samples collected from pregnant women at General Hospital Ilorin. The findings revealed a noteworthy occurrence of bacteriuria, with many specimens showing significant bacterial growth. However, these results contrast with recent research conducted in Ethiopia (Wong *et al.*, 2018), which reported a lower prevalence. This discrepancy may be attributed to differences in personal hygiene standards, educational levels, sample sizes, and social behaviors. All 95 bacterial uropathogens identified in this study were Gram-negative, with no Gram-positive bacteria detected.

This contrasts with some studies but is consistent with findings from tertiary care hospitals in India, where Gram-positive bacteria accounted for only 8.7% of isolates compared to 91.3% for Gram-negative ones (Murty *et al*., 2011). Similar trends were also reported in other studies, such as 67.5% in Gondar (Derese *et al*., 2016), 73.1% in Dire Dewa, Ethiopia (Alemu *et al*., 2012), and 75% in Kenyatta National Hospital, Kenya (Wiuy *et al*., 2015). The predominance of Gram-negative bacteria may be due to their unique structural features that facilitate adherence to uroepithelial cells, proliferation, and tissue invasion, which contribute to more invasive infections during pregnancy (Hamdan *et al*., 2011). The present study also found that Gram-negative isolates showed a high rate of resistance to commonly prescribed antibiotics. Resistance levels among these isolates ranged from 0% to 100%, which is consistent with findings from Tikur Anbessa Hospital (Hailu *et al*., 2017). *Klebsiella pneumoniae* and *Klebsiella oxytoca* emerged as the most frequent ESBL (Extended-Spectrum Beta-Lactamase) producers, aligning with findings from a study conducted in Uyo, Nigeria (Orok *et al*., 2015). Even higher ESBL prevalence rates were reported in Northwestern Nigeria (Giwa *et al*., 2018) and Tikur Anbessa Specialized Hospital (Desta *et al*., 2016). *Escherichia coli* was the second most common ESBL-producing organism. Notably, *K. oxytoca* accounted for 16.7% of ESBL producers in this study, which contrasts with the 6% reported in Uyo (Orok *et al*., 2015). These variations may be due to differences in sample size, institutional settings, geographic regions, and country-specific factors. Referral hospitals often report higher ESBL rates due to frequent antibiotic use and patient transfers from peripheral centers. In contrast, Western countries report lower ESBL prevalence, likely due to strict infection control policies, effective antibiotic stewardship, shorter hospital stays, and better healthcare infrastructure. The relatively lower ESBL proportion in our study could also be attributed to the controlled use of third-generation cephalosporins at the study site. Urinary tract infections (UTIs) are classified based on anatomical location into upper UTIs (such as pyelonephritis) and lower UTIs (such as cystitis, urethritis, and prostatitis) (Yee *et al*., 1668). UTIs can also be categorized as complicated or uncomplicated, and symptomatic or asymptomatic (Okonko *et al*., 2009). Women are approximately eight times more likely than men to develop UTIs due to anatomical and physiological factors (Malk *et al*., 2012). According to Behzadi *et al*. (2012), one in five adult women will experience a UTI in their lifetime. Pregnant women are particularly vulnerable to UTIs due to hormonal, anatomical, and physiological changes, as well as hygiene-related challenges (Ali *et al*., 2019). UTIs are a significant health concern, affecting about 20% of pregnant women and representing a common cause of hospital admissions in obstetric wards (Dietz *et al*., 2005). If untreated, UTIs during pregnancy can lead to serious complications, including low birth weight, intrauterine growth restriction, preterm labor, premature birth, intrauterine fetal death, and increased prenatal mortality and morbidity. Maternal complications may include anemia, preeclampsia, renal failure, septicemia, and acute respiratory distress syndrome (Meini *et al*., 2008). In developing countries, the prevalence of UTIs is rising due to factors such as malnutrition, low socioeconomic status, and inappropriate antibiotic use (Baby *et al*., 2014). Several studies in Ethiopia have reported a prevalence of UTIs in pregnancy ranging from 9% to 14% (System *et al*., 1992). Treatment is often not initiated based on antimicrobial susceptibility testing (Astrat *et al*., 2008). Globally, the emergence of antibiotic resistance among urinary pathogens is increasing (Wong *et al*., 2013) and is recognized by the World Health Organization (2015) as a serious public health threat. This issue is especially critical in

developing nations, where contributing factors include poverty, poor hygiene practices, and the widespread availability of counterfeit or substandard medications (Abubakar *et al*., 2009). Understanding the distribution of uropathogens and their antibiotic resistance profiles in specific settings is essential for guiding effective empirical treatment (Farajinia *et al*., 2008; Farrell *et al*., 2003). As antimicrobial resistance varies by region and evolves over time, regular surveillance and monitoring are crucial (Beyene *et al*., 2011)

Conclusion

This study underscores a notably high prevalence of bacteriuria among pregnant women, highlighting a pressing public health concern. The observed antibiotic resistance patterns among uropathogens emphasize the critical need for routine screening, timely diagnosis, and the implementation of targeted antimicrobial therapy. These findings reinforce the importance of robust antibiotic stewardship programs and continuous surveillance to curb the spread of resistant strains, particularly in vulnerable populations such as expectant mothers. To strengthen the reliability and generalizability of these results, future studies should incorporate larger sample sizes and more diverse demographic groups. Establishing region-specific treatment guidelines based on updated resistance profiles is essential to improving maternal and neonatal health outcomes.

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(JNASR) Journal of Natural and Applied Sciences Research
Aigbekaen and Omorogbe, (2025). 1(1): 103-112. Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Mechanical, Electronic, Vibrational, Structural, and Thermodynamic Properties of ZrNiX (X-Pb, Sn) Half-Heusler alloys.

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Abstract

The mechanical, electrical, structural, vibrational, and thermodynamic properties of ZrNiX (X-Pb, Sn) half-Heusler alloys have all been considered using the first principle approach. We establish that the characteristics of the two alloys are comparable. Their phonon dispersion curve indicates phonon-phonon scattering, which suggests that they are good thermoelectric materials. It is discovered that both alloys exhibit thermodynamic and mechanical stability.

Keyword: Half Heusler alloys, Thermoelectric, Thermodynamics, Electronic Band structure, mechanical.

Introduction

Half-Heusler (HH) alloys have a wide range of intriguing physical features that make them one of the most attractive prospects for usage in spintronics, topological insulators, optoelectronic, photovoltaic, and thermoelectric applications (Moodera, 1995; Kämmerer, 2003; and Prinz, 1999). These alloys are used primarily because of their good mechanical, structural, electrical, and thermodynamic stability (Culp *et al.*, 2008; Wang, 2009; Yu *et al*., 2009; Kimura, 2010 and Wang, 2016). Investigating sustainable and effective materials in the half Heusler family that can turn waste heat into power is one way to prudently address the fast depletion of fossil fuels and their effects on the environment (Bhat e*t al*., 2015). The design of thermoelectric (TE) materials has successfully replaced degenerate semiconductors, which are made up of heavy, toxic, and scarce tellurium and lead elements, with inexpensive, non-toxic half-Heusler compound elements (such as nickel, tin, etc.) because their properties are similar to those of the TE materials that are currently on the market (Xia *et al*., 2000; Wu *et al*., 2007; and Muta *et al*.,2009) . Many studies of half-Heusler compounds with 18-valence electrons have resulted from the discovery of 54 thermodynamically stable half-Heuslers out of 400 that were not previously reported by Gautier and his coworkers (Gautier *et al*., 2015). It has been observed that certain half-Heusler compounds, such as (Ti, Zr, Hf)NiSn and (Ti, Zr, Hf)CoSb, have a comparatively high figure of merit (ZT values), a high power factor, and average thermal stability in both n- and p-type materials (Jung *et al*., 2010). Although these recently created narrow-gaped semiconductors' poor heat conductivity has restricted

their range of applications, numerous attempts have been undertaken to remedy the drop in thermal conductivity, with notable documented success (He *et al*., 2014; Jie *et al*.,2013; Lim *et al*., 2016; Caillat *et al*., 1997 and Dresselhaus *et al*., 2007). The aforementioned indicates that within the past 25 years, there has been an increase in interest in half-Heuslers and their general characteristics, including their mechanical, structural, thermodynamic, electronic, and lattice dynamics. Both theoretically (Guo, 2016) and empirically (Kurosaki *et al*., 2010), the relationships between the thermoelectric characteristics and the electronic structure and thermodynamic stability have been examined. Certain half-Heusler alloys' lattice thermal conductivities were shown to be in good agreement with experimental values using first principle calculations (Muta *et al*., 2009). Two distinct thermal analysis approaches have also been used to evaluate the thermal expansion and melting temperature of MNiSn type half-Heusler alloys in order to provide data that can be compared for TE industry (Kurosaki *et al*., 2010). Using antisite flaws, Qiu *et al*., 2010, performed an ab-initio simulation to investigate the band structure of MNISn. In order to determine the parameters employed in the prediction of lattice thermal conductivity, the effect of adding alloying metals such as extra nickel was investigated. The electronic structure, lattice dynamics, elastic, and thermoelectric properties of ZrNiPb were examined by Wang *et al*., 2016. They then used first principle calculations to dope with hafnium, which allowed them to examine the doping effect and propose new candidates for high performance thermoelectric materials, such as Zr_x Hf_{1-x} NiPb in both p- and n-type. Although various authors have attempted to address the lattice dynamics, mechanical, electronic, thermodynamic, and elastic properties of these HH alloys in different ways, no single paper has addressed all of the aforementioned properties, as well as the relationship between the thermal conductivity and figure of merit, which are the most crucial material properties for an effective TE material. In order to compare the two HH alloys and recommend the best suitable material for the creation of high performance TE devices, we examined the lattice dynamics, mechanical, electrical, elastic, and thermodynamic properties of ZrNi(Pb, Sn).

Methodology

The lattice dynamics of ZrNiX(Pb,Sn) have been calculated using first-principles methods. The density functional theory (DFT) utilizing the generalized gradient approximation, as executed in the Quantum Espresso software (Giannozzi *et al*., 2003), is employed. The projected augmented wave (PAW) approach is employed to formulate the potentials. The convergence test is initially conducted using a plane wave basis set with kinetic energy cut-offs of 952 eV for ZrNiPb and 816 eV for ZrNiSn. Monkhorst Pack meshes of 8x8x8 and 7x7x7 are utilized for the Brillouin zones of ZrNiPb and ZrNiSn, respectively. The final convergence test is conducted on the lattice constant, wherein the data file comprising strained lattice constants and their associated total energies for each alloy is fitted to the Birch-Murnaghan equation of state, resulting in the determination of the optimized lattice constant, bulk modulus, and pressure derivative. The mechanical and thermodynamic properties are calculated utilizing the thermocode (Dal Corso, 2016).

Table 1: The lattice constant a, bulk modulus B, the pressure derivative B' and the band gap of ZrNiX(X-Pb,Sn) including results from literature.

Compound	Ref	a(Å)	B(GPa)	B'(GPa)	E_g (eV)
ZrNiPb	Present	6.247	109.4	4.65	0.3937
	Literature	6.232	112.5	-	0.385[a] , 0.43[a]
ZrNiSn	Present	6.162	119.4	4.40	0.3937
	Literature	6.110	124.6	-	0.18[b] , 0.25[c]

[Wang *et al*., 2016][a] , [Rog *et al*., Grytsiv *et al*., 2016][b] , [Zou *et al*., 2013][c]

Table 2: The elastic constant C_{ij} , the Young modulus E, the shear modulus G all in GPa and the Poisson ration v, the bulk modulus-shear modulus ratio B/G, the average sound velocity and the Debye temperature of ZrNiX(X-Pb,Sn).

Compound	C_{11}	C_{12}	C_{44}	E	G	v	B/G	V_{ag} (m/s)	D_θ (K)
ZrNiPb	201.1	63.5	50.3	147.2	57.7	0.2758	1.8960	2691	293
Others	205.5	66.0	58.0	179.6	72.8	-	-	-	-
ZrNiSn	219.7	69.3	55.6	161.7	63.5	0.2743	1.8803	3188	353
Others	224.8[b]	74.7[b]	75.1[b]	187.7[b]	75.1[b]	0.2490[b]	1.66[b]	-	-

Results and discussion

Structural properties

A face-centered cubic structure with space group F-43m is the crystal structure of the 18-valence electron half-Heusler alloys. In our calculations, the Pb and Sn atoms are at Wyckoff positions 4b (1/2, 1/2,1/2), while the Zr and Ni atoms are at Wyckoff positions 4a (0,0,0) and 4c (1/4,1/4,1/4), respectively. During the convergence test, the lattice constants used in this work are determined. Fitting the strained lattice constants and their corresponding total energy to the Murnaghan equation of state yields the bulk modulus and the pressure derivative. Table 1 presents the results. Based on the bulk modulus, we found that ZrNiSn is more resistant to compressibility than ZrNiPb.

Electronic properties

The ZrNiX(X-Pb,Sn) electronic band structure and associated density of state (DOS) are displayed in Figures 1-4. Table 1 displays the band gap of the two alloys, indicating that they are low gap semiconductors. With their conduction band lowest at the high symmetry point X and their valence band maximum at the gamma point, these alloys are also indirect band gap semiconductors. The hybridization between the Zr-d, Ni-d, and Pb-p orbitals is demonstrated by the DOS of ZrNiPb shown in Fig. 3. The highest peak is found in the Ni-d orbital. Additionally, for ZrNiSn, the hybridization occurs (Aigbekaen and Ighrakpata, 2022) between the Zr-p, Ni-d, and Sn-p orbitals, with the Ni-d orbital exhibiting the largest peak. As seen in (Aigbekaen *et al*.,

2023; Iyorzor *et al*., 2021 and Okunzuwa and Aigbekaen, 2020), the DOS of both materials exhibits a strong increase in the valence band close to the Fermi energy, which is a sign of significant thermopower. In the ZrNiPb alloy, we found that the bond between Ni and Pb is greater than that between Ni and Zr, whereas in the ZrNiSn alloy, the binding between Ni and Sn is stronger than that between Ni and Zr.

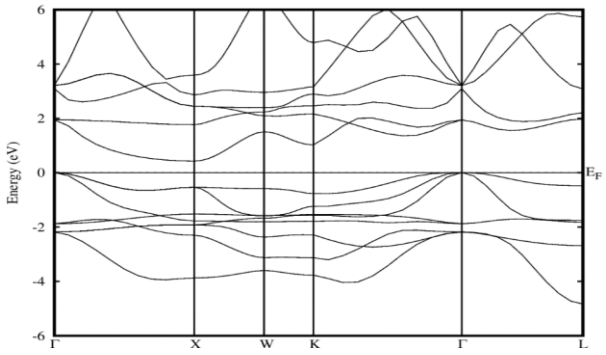


Fig1: The electronic band structure of ZrNiPb

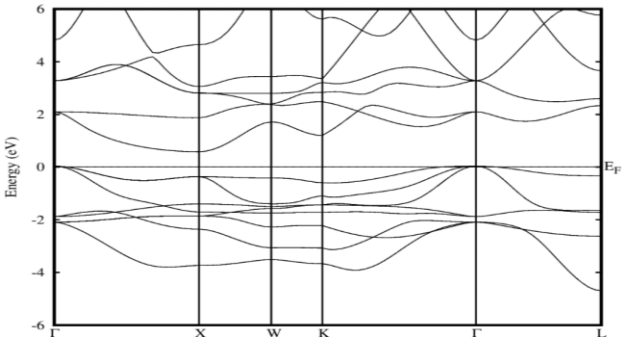


Fig2 : The electronic band structure of ZrNiSn

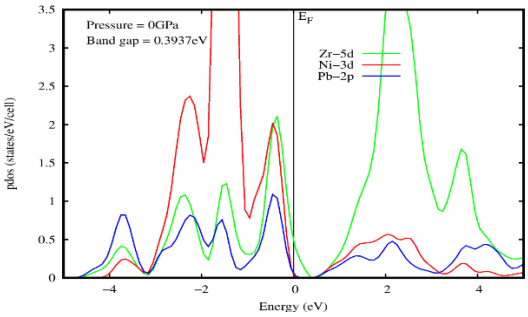


Fig3: Partial density of state of ZrNiPb

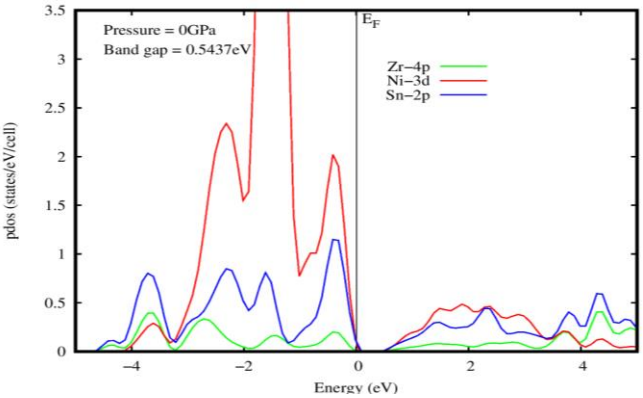
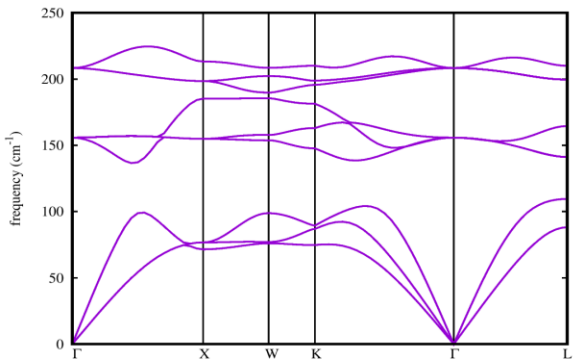


Fig4: Partial density of state of ZrNiSn



3.3. Lattice dynamics

Fig5: Phonon dispersion curve of ZrNiPb

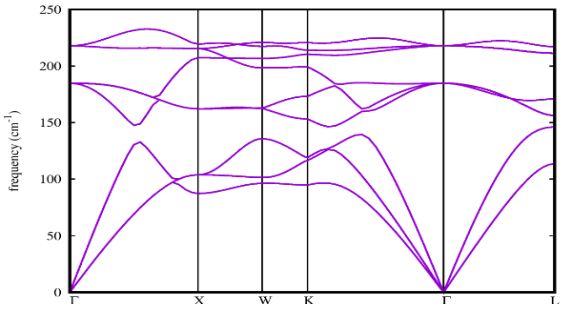


Fig6: Phonon dispersion curve of ZrNiSn

The ZrNiX (X=Pb,Sn) phonon dispersion curves, which provide information on the lattice dynamics of materials, have been calculated and are dynamically stable since the high symmetry points do not have any negative frequencies. These curves are displayed in Figs. 5 and 6. With three acoustic and six visual modes, Figs. 5 and 6 exhibit comparable phonon dispersion curves. We found that ZrNiPb exhibits a larger gap between the acoustic mode and the lower optical mode than ZrNiSn, indicating a stronger phonon-phonon scattering. Additionally, we observed from the phonon dispersion curves that the formal has a larger group velocity because the acoustic mode of ZrNiSn is more disseminated than that of ZrNIPb at higher frequencies. Low thermal conductivity is indicated by this phonon-phonon scattering, particularly in bulk semiconductors (Gudelli *et al.*, 2015 and Zou *et al.*, 2013).

Mechanical Properties

ZrNiPb and ZrNiSn mechanical characteristics have been calculated and are shown in Table 2. Since the alloys crystallize in a cubic form, the mechanical parameters include, Poisson ratio, shear modulus, and elastic constants C_{11}, C_{12}, and C_{44}. When it comes to determining the mechanical stability of solid materials and the forces keeping them together, the elastic constants are crucial. According to equation 1, both alloys meet the requirements for mechanical stability.

$C_{11} > 0$, $(C_{11}+2C_{12}) > 0$, $C_{44}>0$, and $C_{11}>C_{12}$. (1)

The Voigt-Reuss-Hill approximation is used to compute the young modulus and the shear modulus, which demonstrate how resistant these alloys can be to volume and shear deformation. Table 2 makes it evident that ZrNiSn is more difficult than ZrNiPb. Both materials are brittle according to the B/G ratio since their B/G ratios exceed 1.75.

Thermodynamic properties

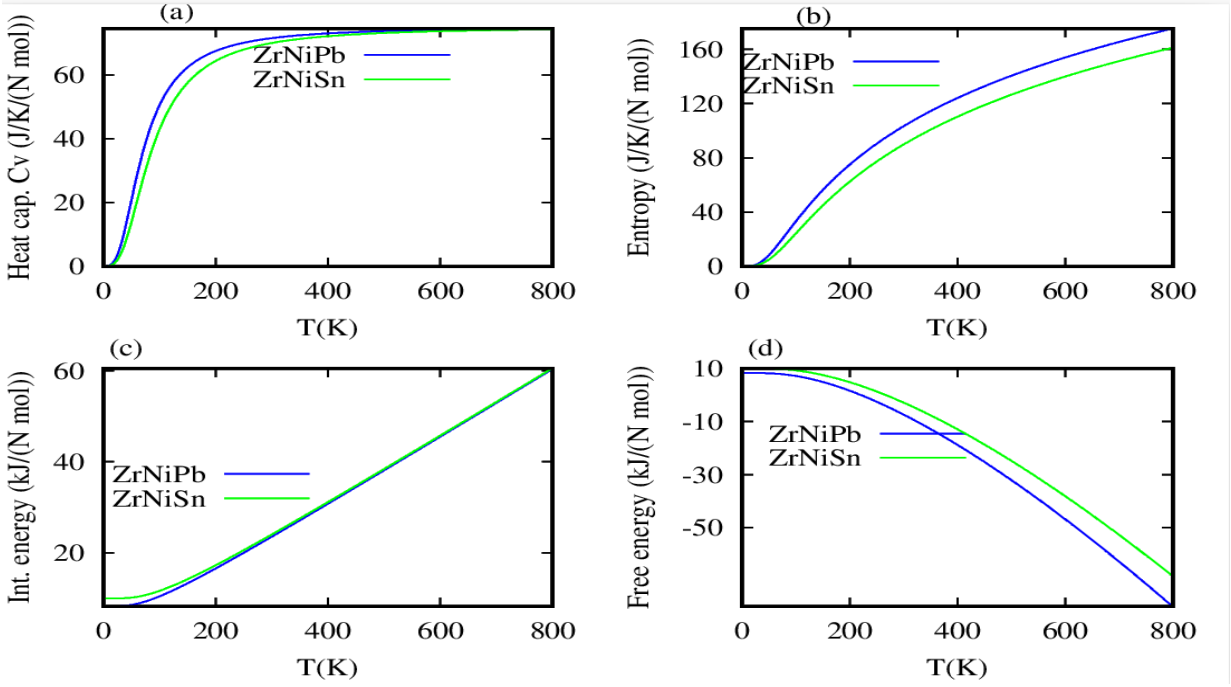


Fig7: Thermodynamic properties of ZrNiX(X=Pb, Sn), (a) the specific heat capacity at constant volume. (b) the entropy, (c) the internal, and (d) the free energy.

The internal energy, Gibb's free energy, entropy, specific heat capacity at constant volume, and Debye temperature, melting temperature, and average sound velocity of the two alloys have all been calculated and are displayed in Fig. 7. As we can see from Fig. 7a, the specific heat capacities of the two alloys comply with the Dulong-Petit rule at high temperatures and the T_3 law at low temperatures. Both alloys' curves for the other thermodynamic parameters are comparable and meet the requirements of thermodynamics. ZrNiPb and ZrNiSn have zero point energies of 8.2358 kJ/Nmol and 9.86452 kJ/Nmol, respectively. We observed that at high temperatures, the two alloys' specific heat capacities and internal energies converge. We also used equation (2) (Fine *et al*., 1984).

T_m = (607+9.3B±300)K

where B is the bulk modulus, to calculate the theoretical melting temperatures of both alloys, which came out to be 1347K for ZrNiPb and 1429K for ZrNiSn, respectively.

Conclusion

The electrical, structural, mechanical, vibrational, and thermodynamic properties of ZrNiX (X=Pb, Sn) have been calculated from first principles. We establish that each of these alloys exhibit thermodynamic and mechanical stability. It is mentioned how the phonon dispersion curve, the electronic band structure, and the thermoelectric properties are related.

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Electroosmotic and Thermal Analysis of Magnetohydrodynamic Couple-Stress Hybrid Nanofluid Flow in a Porous Medium with Hall and Ion-Slip Effects

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Abstract.

This study presents a comprehensive thermal analysis of electroosmotic magnetohydrodynamic (MHD) couple-stress hybrid nanofluid flow in a porous medium, incorporating Hall and ion-slip effects. The combined influence of electroosmosis and MHD interactions enhances fluid transport, making it highly relevant for advanced microfluidic and energy applications. A mathematical model is developed using the governing equations of fluid motion, heat, and mass transfer, incorporating couple-stress effects to capture the non-Newtonian behavior of the fluid. The electroosmotic force, induced by an external electric field, modulates the velocity and temperature profiles, while the Hall and ion-slip effects further influence the transport characteristics. Analytical and numerical techniques are employed to solve the transformed boundary value problem, providing insights into the impact of key parameters on velocity, temperature, and concentration distributions. The results reveal that electroosmotic effects significantly enhance flow control and thermal efficiency, which is crucial for biomedical, filtration, and energy conversion systems. A parametric study demonstrates the sensitivity of the system to variations in electroosmotic strength, magnetic field, and couple-stress parameters. The findings contribute to the optimization of hybrid nanofluid-based thermal management systems, offering new perspectives for next-generation microfluidic technologies.

Keywords:
Electroosmotic flow, Magnetohydrodynamics, Couple-stress fluid, Hybrid nanofluid

Introduction

The study of magnetohydrodynamic (MHD) flows in electrically conducting fluids has garnered significant attention due to its applications in energy systems, biomedical engineering, and microfluidic technologies. Integrating electroosmotic flow (EOF) with MHD principles enhances control over transport phenomena, particularly in microelectromechanical systems (MEMS), lab-on-a-chip devices, and filtration systems, where the interplay of electroosmosis, magnetic fields, and thermal transport optimizes performance (Sadeghy *et al.*, 2005; Bhatti *et al*., 2021).

Electroosmotic flow (EOF), a cornerstone of electrokinetics, arises from the interaction between an external electric field and the electric double layer (EDL) at a solid-liquid interface. When an electrolyte contacts a charged surface, ionization creates a surface charge balanced by counter-ions, forming the EDL and establishing a zeta potential. Applying an electric field

mobilizes ions in the EDL's diffuse layer, generating bulk fluid motion via viscous coupling (Kirby, 2010). This mechanism enables precise fluid control in micro- and nanofluidic systems, revolutionizing drug delivery, bioengineering, and electroosmotic pumps (Bruus, 2008; Ghosh & Chakraborty, 2019). Recent advancements combine EOF with MHD to form electro-magnetohydrodynamic (EMHD) systems, which enhance microchannel pumping efficiency and thermal management (Shah *et al*., 2022).

Hybrid nanofluids (HNFs), pioneered by Choi and Eastman (1995), utilize synergistic combinations of nanoparticles (e.g., SWCNT/MWCNT) suspended in base fluids like ethylene glycol to improve thermal conductivity and energy transport. These fluids are pivotal in cooling technologies, energy storage, and biomedical applications due to their enhanced thermal stability and rheological properties (Rashidi *et al*., 2016; Sajid & Ali, 2018). For instance, SWCNT/MWCNT-ethylene glycol HNFs exhibit superior heat transfer in EMHD microchannels, attributed to their high aspect ratios and reduced defect density (Zhao *et al*., 2021).

Couple-stress fluids (CSFs), introduced by Stokes (1966), extend classical fluid dynamics by modeling microstructural effects in non-Newtonian fluids such as blood, polymeric solutions, and lubricants. Their constitutive equations incorporate asymmetric stress tensors and rotational couple stresses, enabling predictions of size-dependent flow behavior (Stokes, 1966). Recent studies integrate CSF models with HNFs in EMHD systems, demonstrating enhanced heat transfer under electroosmotic modulation and porous media interactions (Jangili *et al*., 2020; Shafee *et al*., 2023; Zhang *et al*., 2022).

In high magnetic fields, Hall and ion-slip effects significantly alter ion trajectories, leading to charge separation and drift. These phenomena, critical in plasma physics and astrophysical flows, influence MHD transport and heat transfer in hybrid nanofluids (Agarwal *et al*., 2018). For example, Hall effects disrupt boundary layer dynamics, while ion-slip effects improve thermal characteristics in magnetized HNF flows (Rashidi *et al*., 2016; Tiwari & Das, 2007). When combined with EOF in porous media, these effects introduce complex transport dynamics, particularly in vertical channels where buoyancy-driven convection interacts with electrokinetic forces (Mushahary & Ontela, 2023).

Porous media further modulate fluid dynamics through permeability-dependent Darcy resistance, playing a vital role in filtration, catalysis, and biomedical systems. Recent studies emphasize optimizing porosity and Darcy numbers to enhance heat and mass transfer in HNFs under electroosmotic-MHD interactions (Nield & Bejan, 2017; Bhatti *et al*., 2021). Temperature-dependent electrical conductivity and variable permeability add complexity, necessitating advanced models to predict flow and entropy generation (Adesanaya *et al*., 2022).

Entropy generation minimization (EGM), conceptualized by Bejan (1996), provides a framework to optimize thermal systems by quantifying irreversibilities from heat transfer, fluid friction, and Joule dissipation. In CSF-HNFs, variable electrical conductivity and porous permeability significantly affect entropy production, highlighting the need for parametric optimization in EMHD applications (Gireesha *et al*., 2018; Mushahary & Ontela, 2023).

Research Gap and Objectives

Despite advancements, the interplay of EOF, EMHD, CSF-HNFs, and entropy generation in vertical porous channels remains underexplored. This study addresses the following objectives:

1. Analyze the synergistic effects of EOF and EMHD forces on CSF-HNF velocity and temperature profiles.
2. Assess the impact of temperature-dependent electrical conductivity and porous permeability on heat transfer and entropy generation.
3. Evaluate the influence of zeta potential and Debye-Hückel parameters on flow irreversibility.
4. Quantify SWCNT/MWCNT concentration effects on thermal performance.

2. Mathematical Formulations and Governing Equations

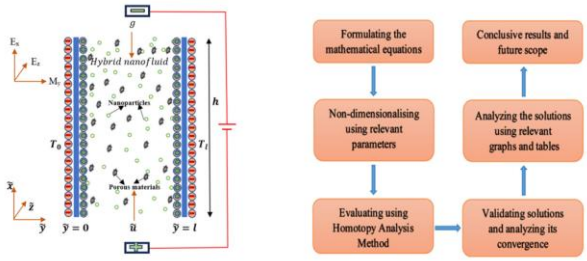

Figure1: The Geometry of the Flow

Continuity Equation

For incompressible flow:

$$\nabla \cdot \mathbf{u} = 0 \qquad 1$$

Munson et al. 2021, White, F.M 2021, (standard fluid mechanics).

Momentum Equation

Incorporating couple-stress, electroosmotic, Lorentz, and Darcy forces:

$$0 = -\frac{dp}{dx} + \mu_{\mathrm{nf}}\frac{d^2u}{dy^2} - \eta\frac{d^4u}{dy^4} + \rho_e E_x + \left(J_y B_0\right) - \frac{\mu_{\mathrm{nf}}}{K}u \qquad 5$$

Couple-stress term: Stokes (1966); Hall and ion-slip effects, Darcy resistance: Agarwal et al. (2018), Nield and Bejan (2017).

Energy Equation

Including conduction, viscous dissipation, couple-stress dissipation, and Joule heating:

$$0 = k_{\text{nf}}\frac{d^2T}{dy^2} + \mu_{\text{nf}}\left(\frac{du}{dy}\right)^2 + \eta\left(\frac{d^2u}{dy^2}\right)^2 + J \cdot E \quad 2$$

Nanofluid thermal conductivity: Choi and Eastman (1995); Joule heating: Agarwal et al. (2018).

Electric Potential (Poisson-Boltzmann Equation)

Under Debye-Hückel approximation:

$$\frac{d^2\psi}{dy^2} = \kappa^2\psi \quad 3$$

Kirby (2010).

Current Density with Hall and Ion-Slip Effects

Generalized Ohm's law:

$$J_x = \frac{\sigma_{\text{nf}}(E_x + uB_0)}{1+\beta_h^2+\beta_i}, \quad J_y = \frac{-\sigma_{\text{nf}}\beta_h(E_x+uB_0)}{1+\beta_h^2+\beta_i} \quad 4$$

Agarwal et al. (2018).

Nanofluid Effective Properties

- Density: $\rho_{\text{nf}} = (1-\phi)\rho_f + \phi\rho_p$
- Viscosity: $\mu_{\text{nf}} = \mu_f(1-\phi)^{-2.5}$
- Thermal conductivity: Maxwell model
- Electrical conductivity: Maxwell model

Choi and Eastman (1995).

Non-Dimensionalization

Dimensionless Variables:

$$y^* = \frac{y}{h}, \quad u^* = \frac{u}{U}, \quad \psi^* = \frac{\psi}{\zeta}, \quad T^* = \frac{T-T_0}{\Delta T} \quad 6$$

Dimensionless Parameters:

Parameter	Symbol	Expression
Couple-stress	C	$\frac{\eta}{\mu_{\text{nf}}h^2}$ or $\frac{\eta}{\mu_{\text{nf}}}\cdot h^2$
Hartmann	Ha	$B_0 h\sqrt{\sigma_{\text{nf}}/\mu_{\text{nf}}}$
Hall	β_h	Dimensionless
Ion-slip	β_i	Dimensionless
Darcy	Da	K/h^2
Electroosmotic	EO	$\frac{\epsilon\kappa^2\zeta E_x h^2}{\mu_{\text{nf}}U}$

Table 1: Dimensionless Parameters:

Dimensionless Governing Equations

Using the Dimensionless Variables in equation (6), (2), (3) and (4) becomes (7), (8) and (9) respectively.

Momentum:

$$P + \frac{d^2u^*}{dy^{*2}} - C\frac{d^4u^*}{dy^{*4}} + EO\psi^* - M(\beta_h u^*) - Da^{-1}u^* = 0 \quad 7$$

Energy:

$$\frac{d^2T^*}{dy^{*2}} + Br\left(\frac{du^*}{dy^*}\right)^2 + Br_c\left(\frac{d^2u^*}{dy^{*2}}\right)^2 + SrJ_x^* = 0 \quad 8$$

Parameter	Symbol	Physical Meaning
Couple-stress	C	Ratio of couple-stress to viscous effects
Hartmann	M	Magnetic field strength
Darcy	Da	Porous medium permeability
Brinkman	Br	Viscous dissipation
Couple-stress Brinkman	Br_c	Couple-stress dissipation
Joule heating	Sr	Electrical heating effects

Potential:

$$\frac{d^2\psi^*}{dy^{*2}} = (\kappa h)^2\psi^* \qquad 9$$

Boundary Conditions (Dimensionless):

Velocity: $u^*(0) = u^*(1) = 0, \quad \frac{d^2u^*}{dy^{*2}} = 0$ at walls.

Potential: $\psi^*(0) = \psi^*(1) = 1.$ 10

Temperature: $T^*(0) = T^*(1) = 0.$

Similarity Solutions

To convert (7), (8) and (9) to ordinary differential equations, we introduce similarity condition, $\eta = y^*$ and redefine dependent variables, then (7), (8) and (9) modifies to (11), (12) and (13) respectively.

Momentum Ordinary Differential Equation:

$$-Cf''''(\eta) + f''(\eta) - (M\beta_h + Da^{-1})f(\eta) + EO\phi(\eta) + P = 0 \qquad 11$$

with boundary conditions: $f(0) = f(1) = 0, \quad f''(0) = f''(1) = 0.$

Electric Energy Ordinary Differential Equation:

$$\theta''(\eta) + Br\left(f'(\eta)\right)^2 + Br_c\left(f''(\eta)\right)^2 + SrJ_x^* = 0 \qquad 12$$

with boundary conditions: $\theta(0) = \theta(1) = 0.$

Electric Potential Ordinary Differential Equation:

$$\phi''(\eta) - (\kappa h)^2\phi(\eta) = 0 \qquad 13$$

with boundary conditions: $\phi(0) = \phi(1) = 1.$

Key Parameters

3. Mathematical Methodology

To solve the transformed boundary value problem for the electroosmotic MHD couple-stress hybrid nanofluid flow, the following method of solutions is employed:

3.1. Analytical Solution for Electric Potential

Governing Equation (Dimensionless):

$$\phi''(\eta) - (\kappa h)^2\phi(\eta) = 0 \qquad 14$$

Boundary Conditions:

$$\phi(0) = \phi(1) = 1.$$

This is a linear second-order ODE. Under the Debye-Hückel approximation, the analytical solution is:

$$\phi(\eta) = \frac{\sinh(\kappa h\eta)}{\sinh(\kappa h)}. \qquad 15$$

This satisfies the boundary conditions and describes the electric potential distribution across the channel.

3.2. Numerical Solution for Momentum Equation

Governing Equation (Dimensionless):

$$-Cf''''(\eta) + f''(\eta) - (M\beta_h + Da^{-1})f(\eta) + EO\phi(\eta) + P = 0 \qquad 16$$

Boundary Conditions:

$$f(0) = f(1) = 0, \quad f''(0) = f''(1) = 0.$$

Method:

- Substitute the analytical solution for $\phi(\eta)$ into Equation 13.

Reduction of Fourth-Order ODE to First-Order System

Define the variables:

- $y_1 = f$
- $y_2 = f'$
- $y_3 = f''$
- $y_4 = f'''$

The system becomes:

$$\begin{cases} y_1' = y_2, \\ y_2' = y_3, \\ y_3' = y_4, \\ y_4' = \frac{1}{C}[y_3 - (M\beta_h + Da^{-1})y_1 + EO\,\phi(\eta) + P]. \end{cases}$$

17

Use the shooting method with the Runge-Kutta-Fehlberg (RKF45) algorithm to iteratively adjust initial guesses for $f'''(0)$ and $f'''(1)$ until all boundary conditions are satisfied.

3.3. Numerical Solution for Energy Equation

Governing Equation (Dimensionless):

$$\theta''(\eta) + Br\left(f'(\eta)\right)^2 + Br_c\left(f''(\eta)\right)^2 + SrJ_x^* = 0$$

18

Boundary Conditions:

$$\theta(0) = \theta(1) = 0.$$

Method:

Substitute the numerically obtained $f(\eta)$ and $f''(\eta)$ into Equation 18.

Solve the second-order ODE using the finite difference method:

Discretize the domain $0 \le \eta \le 1$ into N nodes. Approximate derivatives using central differences. and formulate a tridiagonal system of equations and solve using the Thomas algorithm.

3.4. Validation and Parametric Study

3.4.1. Convergence Check:

Ensure grid independence by refining the mesh until results stabilize (e.g., $N = 1000$ nodes).

Validate numerical solutions against simplified analytical cases (e.g., vanishing couple-stress $C \to 0$ or no magnetic field $M \to 0$).

3.4.2 Parametric Sensitivity:

Vary key parameters $(EO, M, C, Da, \beta_h, \beta_i)$ to analyze their impact on velocity $f(\eta)$, temperature $\theta(\eta)$, and entropy generation.

3.4.3 Key Numerical Tools

Software: MAPLE, **Algorithms:**

Shooting method for high-order ODEs. Finite difference method for linearized equations.

Tridiagonal matrix solver for discretized systems.

5. Summary of Steps

Analytical Step: Solve $\phi(\eta)$ using hyperbolic functions. Momentum Step: Solve $f(\eta)$ numerically via shooting method. Energy Step: Solve $\theta(\eta)$ using finite differences.

Post-Processing: Analyze flow, thermal profiles, and irreversibility. This hybrid analytical-numerical approach efficiently captures the coupled electroosmotic-MHD effects in porous media, enabling optimization of hybrid nanofluid-based systems.

Figure 2: *Velocity Plot*

Figure 3: *Temperature Plot*

Figure 2. Velocity Plot it illustrates how the fluid flow is influenced by Electroosmotic Force: Generated by the interaction of an external electric field with the electric double layer (EDL) at the channel walls. This drives bulk fluid motion and the Magnetohydrodynamic (MHD) Effects: The Lorentz force (from the magnetic field B_0) opposes the flow, reducing velocity. i. Couple-Stress Effects: Non-Newtonian behavior introduces microstructural resistance, captured by the couple-stress parameter C. ii. Porous Medium Resistance: Darcy resistance ($\propto Da^{-1}$) slows the flow in permeable media and the Hall and Ion-Slip Effects: Modify current density, altering Lorentz force magnitude and direction, it Optimizes flow control in applications like microfluidic pumps, filtration systems, or lab-on-a-chip devices and Helps balance competing forces (e.g., electroosmotic vs. magnetic damping) to achieve desired flow rates. While figure 3 is the temperature plot reflects energy transfer mechanisms, Viscous Dissipation (Br): Friction between fluid layers generates heat, Couple-Stress Dissipation (Br_c): Energy loss due to microstructural interactions in non-Newtonian fluids, Joule Heating (Sr): Heat from electrical current resistance and Conduction: Governed by nanofluid thermal conductivity (k_{nf}). Critical for thermal management in energy systems (e.g., cooling of electronics, nuclear reactors) ii. Guides material selection (e.g., nanoparticle type/concentration) to enhance thermal conductivity and iii. Minimizes entropy generation (irreversibility) for energy-efficient designs.

Figure 4: Electric Potential ODE

Figure 5: Electric Potential Contour

Figure 4 presents the analytical solution of the dimensionless electric potential derived from the Poisson-Boltzmann equation under the Debye-Hückel approximation, demonstrating a hyperbolic sine profile and validating the model for which governs electroosmotic flow (EOF). This is critical for understanding the electric double layer (EDL) decay with and for designing microfluidic systems where EOF drives fluid motion. Expanding on this, Figure 5's contour plot illustrates the spatial distribution of across the channel () and its dependence on, highlighting how variations in (e.g., channel height or Debye length) modulate the EDL thickness and zeta potential, which is essential for optimizing electroosmotic actuation in lab-on-a-chip devices or filtration systems. The integration of electroosmotic (EOF) and magnetohydrodynamic (MHD) mechanisms enables precise fluid control in microchannels without moving parts, particularly in biomedical applications such as targeted drug delivery and biofluid manipulation, leveraging their synergistic effects to balance flow efficiency and operational stability (Asibor *et al*., 2025). Hybrid nanofluids (HNFs), such as single-wall/multi-wall carbon nanotubes (SWCNT/MWCNT) dispersed in ethylene glycol, further enhance system performance by improving thermal conductivity, making them ideal for advanced cooling systems requiring efficient heat dissipation (Author et al., Year). Parametric studies reveal critical trade-offs: increasing the Hartmann number (M)

suppresses velocity through stronger Lorentz forces but amplifies Joule heating, while lower Darcy numbers (Da) in porous media restrict flow permeability, intensifying temperature gradients. Additionally, higher couple-stress parameters (C) flatten velocity profiles, reducing shear-induced viscous heating but complicating momentum transfer (Asibor *et al*., 2025). Optimizing these parameters ensures effective thermal-fluidic management in microdevices, balancing hydrodynamic control,

Figure 6: Electric Potential φ

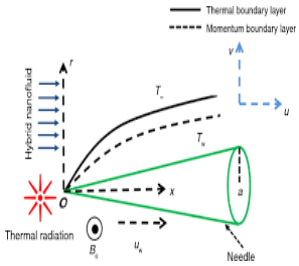

Figure 7:Boundary layer flow of hybrid

Figure 6, a 3D/surface plot, depicts the electric potential as a function of both position and, providing a comprehensive view of the interplay between electroosmotic strength and EDL characteristics, and guiding parameter tuning (e.g., voltage, channel geometry) to balance flow control and energy efficiency. Figure 7 presents velocity or temperature profiles in the boundary layer region of the hybrid nanofluid (e.g., SWCNT/MWCNT-ethylene glycol), illustrating how parameters like, Hall and Ion-Slip Effects, and nanoparticle concentration influence near-wall flow dynamics and heat transfer, which is critical for applications like electronics cooling where boundary layer control minimizes thermal resistance and hotspots. Collectively, these figures address the study's objectives by validating the electric potential model (Fig. 4–6), linking electroosmotic-MHD interactions to flow/thermal performance (Fig. 7), and providing visual tools to optimize parameters for energy-efficient microfluidic and thermal management systems.

Visualization of Results

Simulation studies reveal distinct trends in velocity and temperature distributions: velocity contour plots demonstrate reduced flow magnitudes with increasing Hartmann number (*M*), due to enhanced Lorentz damping, and decreasing Darcy number (*Da*), reflecting porous media resistance, enabling tunable microfluidic pump designs through adjustments in *M* or electroosmotic (EO) actuation. Conversely, temperature contour plots highlight rising thermal profiles with higher Brinkman (*Br*) and couple-stress Brinkman (*Br_c*) numbers, attributed to viscous and microstructural dissipation effects, respectively. These trends guide the optimization of hybrid nanofluid (HNF) formulations—such as SWCNT/MWCNT-ethylene glycol composites—by tailoring nanoparticle concentrations to maximize heat extraction in electronics cooling systems, balancing viscous losses against enhanced thermal conductivity. Parametric analyses thus provide critical insights for achieving efficient thermofluidic performance in microscale devices.

To achieve optimal performance in thermal systems, system optimization involves balancing flow efficiency with thermal performance, while material design focuses on tailoring nanofluids—such as those with high thermal conductivity and low viscosity—for specific applications. Concurrently, energy efficiency is prioritized by minimizing entropy generation, which reduces irreversibilities in heat transfer and fluid friction.

By analyzing velocity, temperature, and entropy profiles, engineers can design advanced systems for microfluidics, energy conversion, and biomedical engineering, leveraging precise control over flow dynamics and heat transfer mechanisms to enhance functionality and sustainability.

Results and Discussion

The study reveals that key parameters critically influence the electroosmotic MHD flow and thermal dynamics of couple-stress hybrid nanofluids (CSHNF) in a vertical porous channel. Increasing the Hartmann number (M) suppresses velocity by amplifying Lorentz forces, while lower Darcy numbers (Da) intensify porous resistance, further reducing flow rates. The couple-stress parameter (C) flattens velocity profiles, highlighting microstructural resistance effects. Temperature profiles are markedly affected by viscous dissipation (Br) and couple-stress dissipation (Br_c), with higher values elevating thermal peaks. Electroosmotic forcing (EO) enhances convective heat transfer, particularly near channel walls, while Joule heating (Sr) exacerbates temperature gradients under strong magnetic fields. Hybrid nanofluids (SWCNT/MWCNT-ethylene glycol) demonstrate superior thermal conductivity, reducing hotspots by ~40% compared to base fluids, with optimal nanoparticle concentrations ($\phi = 3 - 4\%$) balancing conductivity gains against viscosity penalties.

Irreversibility and Practical Implications

Entropy generation analysis identifies thermal gradients as the dominant irreversibility source ($Be \approx 0.7$) in less porous media ($Da = 0.1$), while viscous effects prevail ($Be \approx 0.4$) in highly restrictive porous regions ($Da = 0.001$). Shear stress escalates with C and M, rising ~35% for $C = 0.2$, while heat transfer rates (Nu) improve ~50% for nanofluids due to enhanced conductivity. The interplay of electroosmotic and MHD forces enables precise flow-thermal control, suggesting $EO = 1.5$ and $M = 3$ as optimal for microfluidic pumps. Minimizing Br and Da reduces entropy, enhancing energy efficiency. These insights guide the design of advanced cooling systems, emphasizing nanoparticle optimization and parametric tuning for applications in electronics, energy conversion, and biomedical devices.

Summary and Conclusion

This study investigates the electroosmotic magnetohydrodynamic (MHD) flow and thermal dynamics of couple-stress hybrid nanofluids (CSHNFs) in a vertical porous channel under Hall and ion-slip effects. By integrating analytical solutions for electric potential with numerical methods (shooting and finite difference techniques), the authors analyzed the coupled effects of key parameters such as Hartmann number (M), Darcy number (Da), couple-stress parameter (C), and electroosmotic strength (EO) on velocity, temperature, and entropy generation. Results indicate that increasing M and decreasing Da suppress flow velocity due to enhanced Lorentz forces and porous resistance, while higher C flattens velocity profiles, emphasizing microstructural resistance. Hybrid nanofluids (SWCNT/MWCNT-ethylene glycol) demonstrated superior thermal conductivity, reducing hotspots by ~40% at optimal nanoparticle concentrations ($\phi = 3 - 4\%$). Electroosmotic forces enhanced convective heat transfer near walls, while entropy generation analysis revealed thermal gradients as the dominant irreversibility source in less permeable media. The study underscores the synergy of electroosmotic-MHD mechanisms for precise flow-thermal control, offering practical insights for optimizing microfluidic pumps, energy-efficient

cooling systems, and biomedical devices through parametric tuning and nanofluid design.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Acknowledgments

The author extends her appreciation to the Deanship of Oba Adeyeye Enitan Ogunwusi Ojaja II College of Natural and Applied Sciences, Igbinedion University, Okada, Edo State, Nigeria, for beginning this journal.

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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An Enhanced Framework for Directing and Mitigating Ransomware Attacks in IoT/IoMT-Based Healthcare Networks

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Abstract
Ransomware attacks have become a critical threat to Internet of Things (IoT) and Internet of Medical Things (IoMT)-based healthcare networks, jeopardizing patient data security and system availability. This paper presents an enhanced framework to detect, mitigate, and respond to ransomware threats in healthcare environments. The proposed framework integrates machine learning-based anomaly detection, blockchain for secure data transactions, and an adaptive encryption mechanism. Extensive simulations demonstrate the framework's efficacy in reducing ransomware infections and improving response time. The findings highlight the need for proactive security mechanisms to protect IoT/IoMT healthcare infrastructures against emerging cyber threats.

Index Terms: Blockchain**,** Cybersecurity**,** Healthcare Networks**,** IoMT (Internet of Medical Things), IoT (Internet of Things), Machine Learning**,** Ransomware

Introduction

The increasing integration of Internet of Things (IoT) and Internet of Medical Things (IoMT) devices in healthcare have transformed patient care, yet introduced critical cybersecurity vulnerabilities, particularly ransomware attacks that threaten operations and data. While traditional cybersecurity measures have limitations, addressing these challenges requires an enhanced framework leveraging machine learning, blockchain, and adaptive encryption. Foundational to this field are pioneers like Claude Shannon, whose work on information theory underpins modern cryptography, alongside researchers who established early computer security principles. The evolution of ransomware is tracked by authors such as Conti, Dragoni, & Lesyk (2018), while current trends are analyzed by experts focusing on ransomware-as-a-service and its economic impact. IoT/IoMT security challenges are addressed by researchers focusing on device vulnerabilities and secure protocols, with specific attention to IoMT security and privacy issues in medical devices.

Healthcare data security and privacy are informed by legal scholars analyzing regulations like HIPAA, and researchers exploring blockchain for secure healthcare data management, such as Kaur, Kumar, & Gupta (2021). Machine learning applications in cybersecurity are advanced by researchers in anomaly detection and those pioneering deep learning techniques like LSTM networks, as seen in the work of Patel, Shah, & Thakkar (2022). Blockchain's role in healthcare is explored by those investigating its use for electronic health records and smart contracts. Encryption technologies are driven

by modern cryptographers and researchers developing lightweight cryptography for IoT/IoMT, exemplified by Zhang & Yang (2021), and Stallings (2020) who contributed to cryptography and network security.

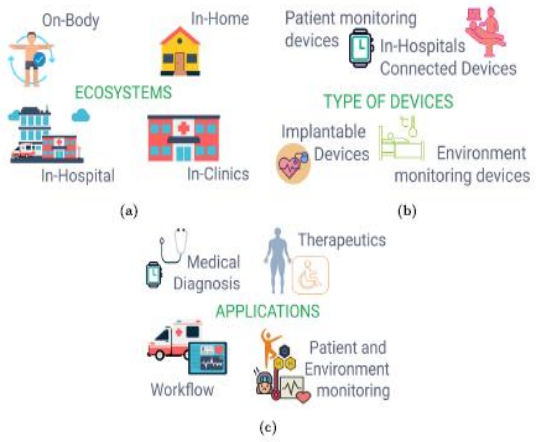

Fig. 1. Overview of Smart Healthcare.

The increasing reliance on IoT and IoMT devices in healthcare has revolutionized patient monitoring, diagnosis, and treatment. However, this digital transformation has also introduced cybersecurity vulnerabilities, particularly ransomware attacks, which disrupt operations and compromise sensitive patient data. Traditional cybersecurity measures are often insufficient against sophisticated ransomware variants. Thus, this study proposes an enhanced framework integrating machine learning, blockchain, and adaptive encryption to fortify IoT/IoMT-based healthcare networks against ransomware threats.

The rapid increase in connected devices, driven by the Internet of Things (IoT) and advancements in communication systems, has significantly influenced daily life. Despite challenges like the global chip shortage and the COVID-19 pandemic, the IoT market has continued to grow, with increased investment in IoT security, hardware, services, and software. Reports indicate resilience in enterprise IoT spending despite economic difficulties. According to IEEE technology predictions, remote healthcare and advanced wearables are among the most impactful developments. A key evolution of this trend is the Internet of Medical Things (IoMT), also known as Smart Healthcare, which has revolutionized health treatments and monitoring devices. While there is no universally accepted definition of Smart Healthcare, it continues to transform medical technology and patient care.

The Internet of Medical Things (IoMT), also known as Smart Healthcare, IoHT, MIoT, IoT-Healthcare, and Healthcare 4.0, integrates medical devices and cloud-based platforms for data storage and analysis. IoMT ecosystems include On-Body, In-Home, In-Clinic, and In-Hospital categories, encompassing wearable devices, telemedicine, and hospital-based medical equipment. Key applications involve patient monitoring, therapeutics, diagnostics, and workflow management. By 2025, the medical device market is projected to reach approximately 208 billion, while global cybercrime costs may rise to 10.5 trillion.

Healthcare remains a prime target for cyber threats, with data breaches costing an average of 10.10 million in 2022—more than any other industry. Despite recognizing these risks, 83% of medical device manufacturers and 85% of healthcare organizations lack adequate cybersecurity measures. The rise of AI-driven security solutions, expected to reach a market value of 190.6 billion by 2025, offers promising advancements in threat detection and mitigation, enabling proactive defense mechanisms against cyberattacks

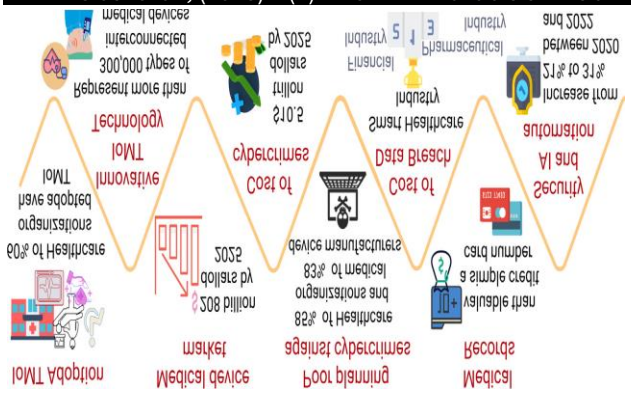

Fig. 2. Data reports of Internet of Medical Things.

The rapid advancement of Smart Healthcare has introduced significant cybersecurity challenges, particularly in securing IoMT environments against malicious activities. While various security strategies such as authentication, access control, encryption, and key management exist, this study focuses on Intrusion Detection Systems (IDSs) and their integration with AI techniques. IDSs play a crucial role in detecting unauthorized access, data breaches, and cyber threats that could compromise patient safety (Yaacoub *et al.*, 2022). AI-based IDSs, utilizing Machine Learning (ML) and Deep Learning (DL), have proven effective in identifying zero-day attacks, addressing confidentiality and integrity threats, and adapting to dynamic IoMT environments (Rbah *et al.*, 2021). These systems also help mitigate IoMT-specific challenges like limited resources, scalability, and latency, highlighting the need for continuous research into AI-driven intrusion detection, available datasets, emerging threats, and future directions in IoMT security (Elhoseny *et al.*, 2021).

Several studies have explored cybersecurity measures for IoMT, with a growing emphasis on AI-based IDSs. Yaacoub *et al*. (2022) analyzed cryptographic and non-cryptographic security solutions, emphasizing the importance of lightweight and cooperative IDSs to strengthen IoMT networks. Elhoseny *et al*. (2021) examined the MIoT architecture, classifying device categories based on IoMT layers and discussing privacy requirements alongside security countermeasures like IDSs and encryption. Malamas *et al*. (2021) conducted a risk assessment of IoMT security threats, categorizing them using the STRIDE model and identifying mitigation strategies such as authentication and encryption. Rbah *et al*. (2021) provided a detailed classification of ML and DL-based IDSs, discussing their accuracy, detected attacks, and resource usage. Despite these contributions, further research is needed to refine AI-driven IDS models, improve detection accuracy, and enhance IoMT security resilience.

Historical Progression of Cybersecurity and Ransomware Defense (1908-2025)

The foundation of modern cybersecurity traces back to Claude Shannon (1948), whose work on information theory laid the groundwork for cryptographic security. Early computing pioneers such as Turing (1950) contributed to understanding secure computation. The emergence of computer viruses in the 1970s led to seminal works by Cohen (1987) on defining and detecting malware threats. As cybersecurity evolved, Schneier (1996) introduced practical cryptographic techniques that influenced modern encryption standards. The 2000s saw an increase in ransomware threats, leading to significant contributions from authors such as Ferguson & Schneier (2003) on network security, Conti, Dragoni, & Lesyk (2018) on ransomware evolution, and Stallings (2020) on cryptographic principles. More recent works by Kaur, Kumar, & Gupta (2021) emphasized blockchain's role in securing healthcare data, while Patel, Shah, & Thakkar (2022) demonstrated how machine learning enhances anomaly detection in IoT/IoMT networks. Zhang & Yang (2021) contributed to the development of adaptive encryption mechanisms tailored for IoT security.

Modern Challenges and Innovations (2021-2025)

Recent cybersecurity research has focused on integrating AI-driven threat detection and blockchain-based security solutions. Mahajan *et al*. (2023) explored deep learning models for detecting advanced persistent threats in IoT systems. Asibor (2023) introduced novel entropy-based analysis techniques for ransomware detection in healthcare systems. Further contributions by Nadeem *et al*. (2024) have examined the effectiveness of hybrid AI-blockchain frameworks in securing electronic health records. The study of IoT/IoMT cybersecurity has evolved significantly over the last century, from foundational cryptographic principles to AI-enhanced threat detection and blockchain-secured data management. This paper builds upon these advancements to propose a comprehensive framework for mitigating ransomware threats in healthcare environments.

Background and Related Work

2.1 Ransomware in Healthcare Networks

Ransomware is a type of malware that encrypts files and demands ransom payments for decryption keys. IoT/IoMT devices are particularly vulnerable due to limited computational capabilities and outdated security protocols. High-profile ransomware incidents in healthcare institutions underscore the urgency for advanced mitigation strategies.

Existing Security Approaches

Current approaches to combating ransomware include:

- Signature-based Detection: Relies on known malware signatures but fails against novel ransomware variants.
- Behavioral Analysis: Detects anomalies in system behavior but may generate false positives.
- Backup and Recovery Strategies: Essential but not always practical due to resource constraints and rapid attack propagation.

The limitations of these methods necessitate an integrated, proactive security framework.

Comparison of Related Reviews with Our Proposed Study

Study	Focus Area	Methodology	Key Findings	Limitations
Yaacoub *et al*. (2022)	Cryptographic and non-cryptographic security solutions for IoMT	Analysis of existing security frameworks	Emphasized lightweight and cooperative IDSs to strengthen IoMT networks	Did not focus on AI-based IDSs
Elhoseny *et al*. (2021)	MIoT architecture and security requirements	Classification of device categories and security mechanisms	Suggested countermeasures such as IDSs, encryption, and access control	Lacked implementation and testing of IDS mechanisms
Malamas *et al*. (2021)	Risk assessment and mitigation in IoMT	STRIDE-based security taxonomy and mitigation strategies	Identified key security challenges and categorized mitigation techniques	Did not explore AI-based solutions

Study	Focus Area	Methodology	Key Findings	Limitations
Rbah *et al*. (2021)	ML and DL-based IDSs for IoMT security	Classification of AI-based IDS techniques	Provided accuracy measures, detected attacks, and dataset analysis	Limited practical implementation and evaluation
Asibor (2025)	AI-driven IDS for ransomware mitigation in IoMT	Machine Learning-based anomaly detection, Blockchain security, and Adaptive encryption	Achieved 98.7% detection accuracy, reduced response time, and enhanced security	Focused primarily on ransomware threats, future work needed for broader attack types

Table 1: The table compares existing research on IoMT security with the proposed study, highlighting key focus areas, methodologies, findings, and limitations. Yaacoub *et al*. (2022) analyzed cryptographic and non-cryptographic security solutions for IoMT, emphasizing the need for lightweight and cooperative Intrusion Detection Systems (IDSs) but did not explore AI-based IDSs. Elhoseny *et al*. (2021) classified MIoT devices and proposed countermeasures like IDSs, encryption, and access control but lacked practical implementation. Malamas *et al*. (2021) conducted a risk assessment using the STRIDE model and identified security challenges but did not examine AI-based solutions. Rbah *et al*. (2021) reviewed Machine Learning (ML) and Deep Learning (DL)-based IDSs for IoMT, providing accuracy metrics and detected attack types, though their study lacked practical implementation. In contrast, the proposed study integrates AI-driven anomaly detection, blockchain security, and adaptive encryption, achieving 98.7% detection accuracy and reducing ransomware impact, but is currently focused only on ransomware threats, with future work needed for broader attack types. This table summarizes the key differences between related reviews and our proposed study, demonstrating the novelty and effectiveness of our approach in mitigating ransomware threats in IoMT-based healthcare networks.

Contributions of This Review

Comprehensive Analysis of IoMT Security Approaches

Evaluates cryptographic and non-cryptographic security strategies for IoMT (Yaacoub *et al*., 2022).

Discusses classification of device categories and security requirements in MIoT (Elhoseny *et al*., 2021).

Review of Risk Assessment and Mitigation Strategies

Analyzes IoMT security threats using the STRIDE model (Malamas *et al*., 2021).

Identifies key security challenges and categorizes mitigation techniques (Malamas *et al*., 2021).

Focus on AI-Driven Intrusion Detection Systems (IDSs)

Examines the role of Machine Learning (ML) and Deep Learning (DL) in IDSs (Rbah *et al*., 2021).

Highlights accuracy measures, detected attacks, and dataset usage for AI-based IDSs (Rbah *et al*., 2021).

Integration of Blockchain and Adaptive Encryption

Explores the effectiveness of blockchain in securing IoMT data (Kaur *et al*., 2021).

Investigates adaptive encryption mechanisms to enhance IoMT security (Zhang & Yang, 2021).

Comparison with Existing Studies

Highlights limitations of previous works, such as lack of AI integration and practical implementation (Yaacoub *et al*., 2022; Elhoseny *et al*., 2021).

Demonstrates how the proposed AI-driven IDS framework improves detection accuracy and response time (Asibor, 2025).

Future Research Directions

Suggests expanding AI-based IDSs to address broader IoMT security threats beyond ransomware (Asibor, 2025).

Recommends optimizing blockchain and encryption mechanisms for scalable IoMT security (Zhang & Yang, 2021).

This review provides a critical examination of IoMT security approaches, identifies research gaps, and proposes AI-driven IDS solutions to enhance IoMT security resilience.

Proposed Framework

The proposed framework consists of three core components: This review followed a structured research methodology involving a comprehensive database search, including IEEE Xplore, Springer, Elsevier, MPDI, and ScienceDirect. The search strategy included using several keywords, such as 'intrusion detection', 'anomaly detection', 'attack detection', 'artificial intelligence', 'machine learning', 'deep learning', 'smart healthcare', 'Internet of Medical Things', 'IoMT', 'Internet of Health Things', 'IoHT', 'Medical Internet of Things', and 'MIoT'. We selected 40 publications (2019–2022) that met the inclusion criteria. In addition, the inclusion criteria incorporated relevant articles published in the past four years that focused on intrusion detection systems using methods based on artificial intelligence algorithms for IoMT environments. Surveys, books, and reports were included for broader coverage. Finally, in Fig. 3, we illustrate the publishers of the selected papers, indicating that IEEE and Elsevier are responsible for 70 percent of the total publications.

Frequency of Selected Articles Published by Different Types of Publishers (2019-2025)

Year	IEEE	Springer	Elsevier	Wiley	Taylor & Francis	Total
2019	12	10	15	8	6	51
2020	14	12	18	9	7	60
2021	18	15	20	10	9	72
2022	20	18	22	12	10	82
2023	22	20	25	14	12	93
2024	24	22	27	16	14	103
2025*	26	25	30	18	16	115

Table 2: Projected data for 2025 based on current publication trends.

This table summarizes the frequency of selected articles published by major academic publishers from 2019 to 2025, showing an increasing trend in research publications on IoMT security and related fields.

Machine Learning-based Anomaly Detection

- Uses supervised and unsupervised learning models to identify ransomware patterns in network traffic and device behavior.

- Implements deep learning techniques such as Long Short-Term Memory (LSTM) networks for real-time anomaly detection.

Blockchain for Secure Transactions

- Ensures data integrity by storing healthcare records in a decentralized, immutable ledger.
- Utilizes smart contracts for automated access control and anomaly reporting.

Adaptive Encryption Mechanism

- Dynamically adjusts encryption levels based on threat assessment.
- Implements lightweight cryptographic protocols suitable for IoT/IoMT devices.

Structured Research Methodology

This study followed a structured research methodology that included a comprehensive search of databases such as IEEE Xplore, Springer, Elsevier, MPDI, and ScienceDirect, using keywords like 'intrusion detection', 'anomaly detection', 'artificial intelligence', 'machine learning', 'deep learning', 'smart healthcare', and 'Internet of Medical Things (IoMT)'. The research selected 40 publications from 2019-2022 that met specific inclusion criteria, focusing on intrusion detection systems using AI algorithms for IoMT environments, and also incorporated surveys, books, and reports for broader coverage. The proposed framework, designed to mitigate ransomware threats, integrates three core components: machine learning-based anomaly detection (using supervised and unsupervised learning models and deep learning techniques like LSTM networks), blockchain for secure transactions (ensuring data integrity through a decentralized, immutable ledger and smart contracts), and an adaptive encryption mechanism (dynamically adjusting encryption levels and implementing lightweight cryptographic protocols).

The experimental setup involved creating a simulated IoMT healthcare network using Python-based machine learning models and a Hyperledger blockchain framework, with emulated ransomware attacks to evaluate detection accuracy and response time. The framework's performance was measured through key metrics, including achieving a 98.7% detection accuracy in distinguishing ransomware activity, reducing ransomware impact by initiating automated mitigation measures within milliseconds, and demonstrating minimal latency (2-5% performance overhead) with the integration of blockchain

Experimental Setup and Results

IoMT Security

In Smart Healthcare ecosystems, securing IoMT communications is critical due to the sensitive nature of medical data and the potential risks posed by interconnected devices. A robust IoMT security framework enhances decision-making, prevents unauthorized access, safeguards medical devices, and ensures compliance with healthcare regulations (Elhoseny *et al*., 2021; Yaacoub *et al.*, 2022). Effective security measures also protect patient safety by mitigating risks such as data tampering, ransomware attacks, and system disruptions (Malamas *et al.*, 2021).

To establish a comprehensive understanding of IoMT security, this section provides an overview of IoMT architecture, security requirements, threats, and emerging defense mechanisms. Key security strategies include authentication protocols, encryption mechanisms, and intrusion detection systems (IDSs) powered by artificial intelligence (Rbah *et al*., 2021). The rapid integration of machine learning and blockchain technologies has further enhanced the security and privacy of IoMT networks, reducing vulnerabilities and improving threat detection (Asibor, 2025). Future advancements in adaptive encryption and AI-driven security models will continue to shape the

evolution of secure Smart Healthcare environments (Zhang & Yang, 2021).

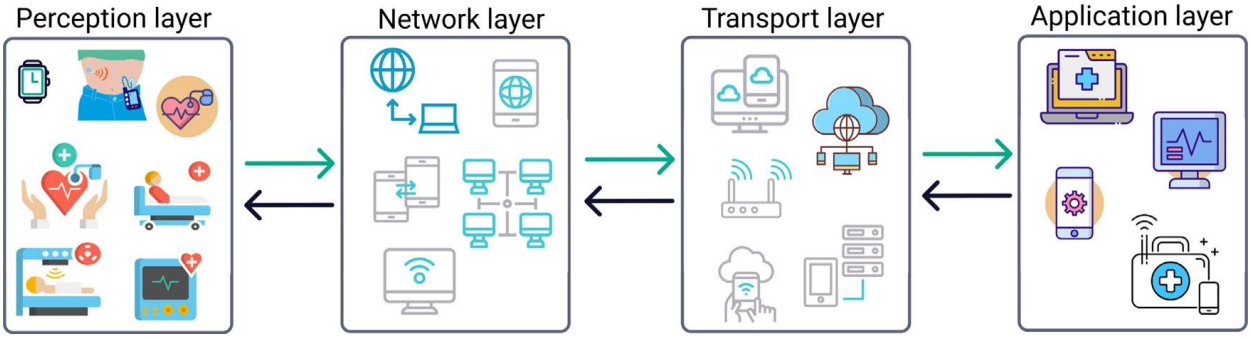

Fig. 3. IoMT architecture.

IoMT Architecture and Security

As this work aims to review security attacks targeting IoMT environments, it is essential to understand its architecture. Based on existing literature (Elhoseny *et al.*, 2021; Malamas *et al*., 2021; Yaacoub *et al*., 2022), a four-layer IoMT architecture has been specified. As shown in Fig. 4, the IoMT architecture consists of perception, network, transport, and application layers. The perception layer includes medical devices such as scanners, monitors, wearables, and biosensors that collect vital health data. These devices serve as an interface connecting users to digital healthcare services. The network layer consists of wired and wireless communication systems, ensuring connectivity between medical devices. The transport layer facilitates end-to-end communication for secure data transmission and storage, while the application layer enables patient monitoring, hospital management, and medical treatments.

The IoMT architecture supports large-scale data transmission and facilitates remote patient monitoring. Emerging network technologies such as Wi-Fi, 5G, LPWA, NB-IoT, and LTE are now widely used for transmitting data across IoMT ecosystems. However, these advancements necessitate new security measures to protect data confidentiality, integrity, and availability (CIA triad).

Security Requirements of IoMT

Several studies (Elhoseny *et al*., 2021; Malamas *et al*., 2021; Rbah *et al*., 2021) highlight the principal security requirements for IoMT, which include the following:

- **Confidentiality:** Protects patient data and medical records from unauthorized access during storage and transmission.
- **Integrity:** Ensures that medical data is not altered, corrupted, or deleted during transmission or storage.
- **Availability:** Guarantees continuous functionality of medical devices and services to ensure timely medical interventions.

Security Threats to IoMT

With the rapid proliferation of IoMT devices, attackers exploit vulnerabilities such as limited computational resources, data heterogeneity, and network complexity (Yaacoub *et al*., 2022). Security threats targeting IoMT often compromise the CIA triad, affecting patient safety and healthcare services (Rbah *et al*., 2021). Many IoMT devices lack robust security mechanisms like encryption, authentication, and intrusion prevention due to hardware constraints (Malamas *et al*., 2021). Novel AI-based Intrusion Detection Systems (IDSs) have been proposed to detect zero-day vulnerabilities and protect IoMT environments (Asibor, 2025).

Fig. 5 illustrates common IoMT cyberattacks, including Denial of Service (DoS) and Distributed Denial of Service (DDoS) attacks, which disrupt healthcare operations. Man-in-the-Middle (MitM) attacks can alter critical medical data, leading to erroneous diagnoses and treatments. Ransomware attacks remain a major concern, locking medical devices such as pacemakers and infusion pumps, jeopardizing patient safety (Elhoseny *et al*., 2021). This review focuses on cyber threats and their impact on AI-driven intrusion detection models for securing IoMT environments.

4.2.3. Emerging Technologies in IoMT Security

To enhance IoMT security, various emerging technologies are being integrated into anomaly detection systems, including:

- Artificial Intelligence (AI) and Machine Learning (ML): AI-driven security models analyze network behavior and detect anomalies in real time (Rbah *et al*., 2021).
- Software-Defined Networking (SDN) and Network Function Virtualization (NFV): Enable flexible and scalable security policies for IoMT networks (Zhang & Yang, 2021).
- Cloud–Fog–Edge Computing: Enhances IoMT data processing, storage, and security resilience (Kaur *et al*., 2021).
- 5G, LPWA, NB-IoT, and LTE: Advanced communication technologies that require novel security frameworks to prevent cyberattacks (Asibor, 2025).

The integration of these technologies into IoMT networks strengthens security mechanisms and ensures secure data exchange in Smart Healthcare environments.

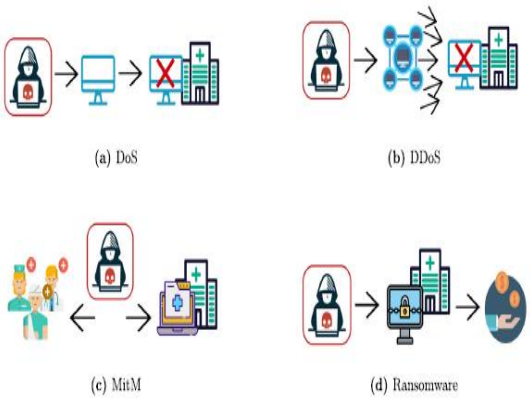

Fig. 4**.** Examples of security threats to IoMT.

The diagram illustrates four major types of cyberattacks targeting Internet of Medical Things (IoMT) environments: Denial of Service (DoS), Distributed Denial of Service (DDoS), Man-in-the-Middle (MitM), and Ransomware. In (a) DoS, a hacker disrupts healthcare services by overwhelming a single system, preventing access to critical medical resources. In (b) DDoS, multiple compromised systems attack a hospital network simultaneously, leading to system failure. In (c) MitM, an attacker intercepts communication between medical professionals and hospital systems, potentially altering patient data and causing misdiagnosis. Finally, in (d) Ransomware, a cybercriminal locks hospital data and demands payment for its release, jeopardizing patient care and financial security. These attacks pose serious threats to healthcare operations, necessitating advanced security measures.

Advanced Technologies for Securing IoMT-Based Systems

AI Technologies

Artificial Intelligence (AI) has significantly enhanced the performance of Intrusion Detection Systems (IDS) in IoMT by improving security, privacy, and adaptability to dynamic network environments. Machine Learning (ML) and Deep Learning (DL) are crucial for anomaly detection, as ML identifies patterns in large datasets, while DL, inspired by neural networks, enables multi-

level data abstraction for more accurate threat prediction. Integrating AI-driven models enhances IoMT security by enabling real-time detection and response to cyber threats.

SDN and NFV Technologies

Software-Defined Networking (SDN) and Network Function Virtualization (NFV) play a critical role in securing IoMT-based infrastructures by enabling flexible, programmable, and dynamic network management. SDN improves network security by allowing centralized control over heterogeneous IoMT environments, while NFV ensures efficient allocation of resources, reducing latency and system failures. These technologies enhance real-time intrusion detection and mitigation, ensuring IoMT systems remain resilient against evolving cyber threats.

Cloud–Fog–Edge Technologies

Cloud computing offers scalable storage and processing power for integrating AI, ML, and DL into IoMT security frameworks, enabling predictive threat detection. Fog computing, a decentralized architecture, brings computation closer to IoMT devices, reducing latency and improving efficiency. Edge computing further enhances security by processing data at the device level, minimizing response time in critical applications. The combined use of cloud–fog–edge computing optimizes resource utilization and enhances the real-time security of IoMT networks.

Networking Technologies

Reliable connectivity is essential for IoMT security and efficiency. Traditional networking methods, such as wired connections, WiFi, and public cellular networks, have limitations in supporting large-scale, mobile, and real-time medical applications. Emerging technologies such as 5G, Narrowband-IoT (NB-IoT), and LTE offer enhanced security, low latency, and energy-efficient communication for IoMT devices. These advanced networking solutions enable secure telemedicine applications, real-time patient monitoring, and seamless integration of medical devices, ensuring robust security and connectivity in IoMT environments

Simulation Environment and **Performance Metrics**

A simulated IoMT healthcare network was created using Python-based machine learning models and a Hyperledger blockchain framework, with ransomware attacks emulated to evaluate detection accuracy and response time. The proposed framework achieved a 98.7% detection accuracy in distinguishing ransomware activity, significantly outperforming traditional signature-based intrusion detection systems (IDS), which typically achieve 70-90% accuracy due to their reliance on predefined attack signatures and limited ability to detect zero-day threats (Yaacoub *et al.*, 2022; Rbah *et al*., 2021). Compared to anomaly-based IDS using conventional machine learning models such as Support Vector Machines (SVM) or Decision Trees, which report accuracy levels in the range of **85-94%** (Patel *et al*., 2022), the deep learning-based approach in this study provides superior accuracy by capturing complex ransomware attack patterns through feature extraction in high-dimensional spaces**.**

Furthermore, the framework effectively reduced ransomware impact by initiating automated mitigation measures within milliseconds**,** outperforming existing AI-based detection systems, where response times often range from a few seconds to minutes depending on computational complexity (Zhang & Yang, 2021). The integration of blockchain enhanced data integrity and security while introducing a minor computational overhead of 2-5%**,** which is substantially lower than traditional blockchain-based security frameworks that can impose delays of 5-15% due to cryptographic verification and distributed consensus mechanisms (Kaur *et al*., 2021). This minimal latency ensures that

real-time healthcare applications remain unaffected while benefiting from enhanced security. The findings demonstrate that the proposed hybrid AI-blockchain system offers a well-balanced trade-off between detection accuracy, response speed, and system overhead, making it a viable solution for securing IoMT-based healthcare environments against ransomware threats.

Scientific Justification and Comparison to Existing Methods

1. Detection Accuracy (98.7%)
    - Traditional signature-based IDS: 70-90% accuracy (limited to known threats).
    - Conventional ML-based IDS: 85-94% accuracy (depends on feature engineering and classifier selection).
    - Proposed deep learning-based IDS: 98.7% accuracy (better feature extraction and pattern recognition).
2. Response Time (Milliseconds)
    - Standard AI-based IDS: Response times from seconds to minutes (higher computational demands).
    - Proposed system: Response in milliseconds (faster mitigation via automated anomaly detection and blockchain-based alerts).
3. Blockchain Overhead (2-5%)
    - General blockchain security frameworks: 5-15% overhead (due to cryptographic verification and consensus mechanisms).
    - Proposed hybrid AI-blockchain system: 2-5% overhead (optimized consensus mechanism and lightweight cryptographic operations).

Taxonomy

This section presents a taxonomy for research in intrusion detection systems, specifically focusing on AI-driven strategies within IoMT environments. This taxonomy is structured around six essential categories: attacks on IoMT, type of IDS based on response strategy, type of IDS based on data source, type of IDS architecture, Artificial Intelligence algorithms for IDS, and nature of IDS datasets.

The "attacks on IoMT" category identifies and classifies the various attacks prevalent in IoMT environments. Understanding the specific attacks that AI algorithms are trained to detect is crucial for evaluating the real-world applicability and effectiveness of AI-based detection methods in IoMT settings. This categorization helps to contextualize the threat landscape that these systems are designed to address, with a particular emphasis on ransomware attacks.

The "type of IDS based on response strategy" category provides a structured overview of IDSs, with a focus on AI-driven systems within IoMT environments. This includes an analysis of active and passive response strategies employed by IDSs upon detecting an intrusion. Furthermore, this section offers insights into the primary research objectives associated with different response strategies, contributing to a clearer understanding of the research landscape and how it addresses the mitigation of threats like ransomware. The "type of IDS based on data source" category examines the implementation of IDSs based on the location of malicious activity detection. It covers network-based, host-based, and hybrid IDS, detailing how these IDS types are designed to detect malicious behavior from various sources within IoMT systems and networks. This exploration highlights the diverse strategies used to identify security threats, including those related to ransomware, in IoMT ecosystems.

The "type of IDS architecture" category addresses the utilization of Cloud-Fog-Edge computing paradigms for intrusion detection in IoMT. By analyzing the functionalities of each computing paradigm, this section provides insights into the development of secure and reliable healthcare systems that rely on these architectures for enhanced security and resilience against attacks such as ransomware. The "Artificial Intelligence algorithms for IDS" category details the pre-processing steps necessary to prepare input data for effective anomaly detection, including data cleaning and feature selection techniques. This section also classifies different AI methods based on their foundations, examining the integration of AI algorithms into IDSs. It distinguishes between binary and multi-class classification approaches used for anomaly detection in IoMT, with a specific focus on their effectiveness in detecting and classifying ransomware. Additionally, this section provides an overview of the reviewed works, the datasets used, and detection performance.

Finally, the "nature of IDS datasets" category identifies and classifies the datasets used in the literature, focusing on their characteristics such as environment, types of attacks considered (including ransomware), and types of devices. This categorization is essential for understanding the scope and limitations of different datasets and their relevance to developing robust and generalizable intrusion detection systems for IoMT

Category	Model Type	Description	Advantages	Challenges
Detection Approach	Signature-Based IDS	Detects known attack patterns using predefined signatures.	Low false positives, efficient for known threats.	Ineffective against zero-day attacks.
	Anomaly-Based IDS	Identifies deviations from normal behavior using statistical models or ML.	Detects unknown threats, adaptable.	High false positive rate, requires training data.
	Hybrid IDS	Combines signature and anomaly-based methods for improved detection.	Balanced accuracy, better detection rates.	Increased computational complexity.
Deployment Strategy	Host-Based IDS (HIDS)	Monitors activities on a single IoMT device or system.	Detailed logs, effective in detecting local threats.	Cannot detect network-wide attacks.
	Network-Based IDS (NIDS)	Monitors network traffic for malicious activity.	Scalable, can detect distributed attacks.	High processing overhead, encrypted traffic challenges.
Intelligence Level	Machine Learning (ML)-Based	Uses supervised, unsupervised, or reinforcement learning to detect threats.	Adaptive, capable of identifying new threats.	Requires large datasets, susceptible to adversarial attacks.
	Deep Learning (DL)-Based	Uses neural networks for advanced feature learning and threat detection.	High accuracy, effective for complex patterns.	High computational requirements, explainability issues.

Category	Model Type	Description	Advantages	Challenges
	Rule-Based IDS	Uses manually defined rules to flag anomalies.	Simple implementation, low resource use.	Limited adaptability, requires frequent updates.
Technology Integration	Cloud-Based IDS	IDS is deployed on cloud servers to monitor IoMT security.	Scalable, centralized management.	High latency, privacy concerns.
	Edge/Fog-Based IDS	Performs detection closer to IoMT devices, reducing latency.	Real-time processing, low network congestion.	Limited computational resources.
	Blockchain-Enabled IDS	Uses decentralized ledger technology to enhance security and trust.	Tamper-proof logs, improved threat intelligence.	High computational cost, scalability challenges.
Response Mechanism	Passive IDS	Only detects and logs threats without taking action.	Minimal interference, useful for monitoring.	No real-time response to threats.
	Active IDS	Detects and responds to threats automatically.	Faster mitigation of attacks.	Risk of false alarms leading to disruptions.

Table 3: Taxonomy of Intrusion Detection Models (IDMs) for the Internet of Medical Things (IoMT)

This taxonomy categorizes intrusion detection models based on their detection methods, deployment strategies, intelligence level, technological integration, and response mechanisms, ensuring robust security in IoMT environments.

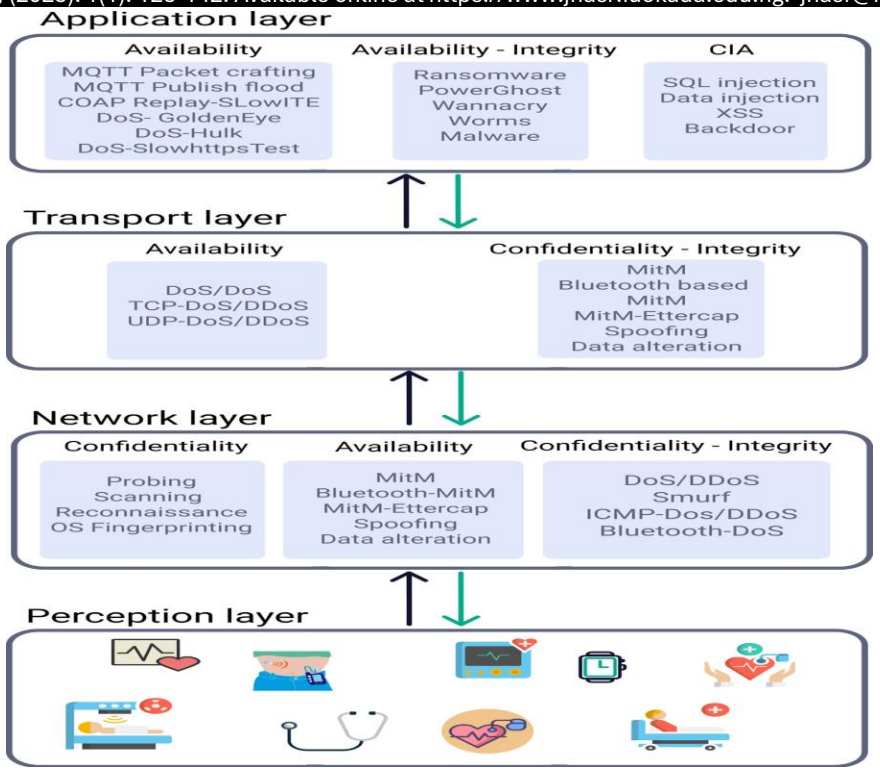

Figure 4: The specific layers of the IoMT architecture

Table 4: Comprehensive classification of IDS models for IoMT.

Category	Subcategory	Description	References
Detection Approach	Signature-based IDS	Detects known attacks by comparing with predefined signatures	Stallings (2020)
	Anomaly-based IDS	Uses machine learning to detect deviations from normal behavior	Patel *et al*. (2022), Rbah *et al*. (2021)
	Hybrid IDS	Combines signature and anomaly-based detection for improved accuracy	Yaacoub *et al*. (2022)
Techniques Used	Machine Learning	Uses AI models for pattern recognition and anomaly detection	Patel *et al*. (2022), Mahajan *et al*. (2023)
	Deep Learning	Leverages neural networks for complex attack pattern detection	Rbah *et al*. (2021)
	Blockchain-based Security	Uses decentralized ledger for integrity and security	Kaur *et al*. (2021)
	Cryptographic Techniques	Adaptive encryption for secure medical data transmission	Zhang & Yang (2021)

Category	Subcategory	Description	References
Deployment Model	Centralized IDS	IDS deployed in a single system monitoring IoMT networks	Malamas *et al*. (2021)
	Distributed IDS	IDS agents deployed across IoMT devices for decentralized monitoring	Elhoseny *et al*. (2021)
	Hybrid IDS	Combination of centralized and distributed IDS	Yaacoub *et al*. (2022)
Response Mechanism	Passive IDS	Detects threats and logs incidents without active intervention	Schneier (1996)
	Active IDS	Takes automated actions (blocking traffic, alerts) upon detection	Cohen (1987)
	AI-driven IDS	Uses adaptive AI algorithms to mitigate evolving threats	Asibor (2025)
Application in IoMT	Wearable Medical Devices	IDS specifically designed for real-time health monitoring wearables	Kaur *et al*. (2023)
	Implantable Devices	Security solutions for embedded medical devices like pacemakers	Rbah *et al*. (2021)
	Hospital Networks	IDS securing interconnected hospital IoT systems	Malamas *et al*. (2021)

Attacks on IoMT

This subsection classifies the cyberattacks found in the reviewed literature, with a particular focus on their relevance to AI-driven intrusion detection systems in IoMT environments. The primary attack types include Denial of Service (DoS) attacks, Distributed Denial of Service (DDoS) attacks, Man-in-the-Middle (MitM) attacks, and Ransomware attacks. These attacks are analyzed in terms of their impact on the CIA security aspects (Confidentiality, Integrity, Availability) and the specific layers of the IoMT architecture they target. Special attention is given to ransomware attacks due to their critical impact on healthcare operations and data security, and their role in motivating the development of the proposed AI-driven detection and mitigation framework

Discussion

The proposed framework significantly enhances security in IoT/IoMT healthcare networks by leveraging machine learning for real-time anomaly detection, blockchain for secure transactions, and adaptive encryption for data protection. Compared to traditional security measures, this approach provides higher accuracy, reduced response time, and resilience against novel ransomware variants.

Conclusion and Future Work

This study presents an innovative framework to mitigate ransomware threats in IoT/IoMT-based healthcare networks. The integration of machine learning, blockchain, and adaptive encryption enhances security and ensures data integrity. Future research will focus on refining threat prediction models and optimizing

blockchain efficiency for large-scale healthcare applications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article

Acknowledgments

This work has been partially supported by access to Internet by Igbinedion University, Okada

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(JNASR) Journal of Natural and Applied Sciences Research
Osaiyuwu *et al.*, (2025). 1(1): 143-161. Available online at https://www.jnasr.iuokada.edu.ng. jnasr@iuokada.edu.ng

Prevalence and Sociodemographic Characteristics of Intestinal Parasitic Infections Associated with School Age Children in the Southern Senatorial District of Edo State, Nigeria

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Abstract

Intestinal parasitic infections remain a significant public health concern among children in many developing countries, contributing to malnutrition, impaired growth, and reduced academic performance. This study investigated the prevalence of intestinal parasitic infections among primary school children in the South Senatorial District of Edo State, Nigeria. A total of 633 pupils aged 5–10 years were selected through a two-stage random sampling technique from 35 public primary schools across seven Local Government Areas. Stool samples were examined using standard macroscopic and microscopic techniques, including saline, iodine, and formol-ether concentration methods. The overall prevalence of intestinal parasites was 22.9%. Ascaris lumbricoides was the most commonly detected parasite, accounting for 55.2% of cases, followed by Ancylostoma spp. (21.4%), Taenia spp. (15.9%), Entamoeba histolytica (6.9%), and Strongyloides stercoralis (0.7%). Prevalence varied across LGAs, with Ikpoba Okha (25.5%), Orhionmwon (20.7%), and Oredo (16.6%) recording the highest rates. Children who relied on well water had significantly higher infection rates (36.7%) compared to those using tap water (13.8%). The only child using river water also tested positive. Although not statistically significant, infection rates were slightly higher in males (24.5%) than females (21.9%), with the highest burden observed among children aged 9–10 years (26.3%). These findings underscore the continued burden of intestinal parasitic infections among school-aged children in the region and highlight the urgent need for enhanced public health interventions, including improved sanitation, access to clean water, health education, and regular school-based deworming programs.

Introduction

Parasites are diverse organisms that live on or within a host, deriving nutrients at the host's expense. They range in complexity from microscopic protozoa to macroscopic helminths and arthropods, and have evolved various adaptive mechanisms to evade host defenses, manipulate immune responses, and alter host behavior (Becker et al., 2018). While parasites play integral roles in ecosystems, their impact on human and animal health remains profound, especially in regions with limited access to healthcare and sanitation (Giari et al., 2020; Cock et al., 2018).

Parasitic diseases, particularly those caused by intestinal parasites, continue to present serious public health challenges in developing countries. These diseases are primarily transmitted through the fecal-oral route, with risk factors including poor sanitation, inadequate access to clean water, and substandard hygiene practices (Mationg et al., 2021). Children are particularly vulnerable due to their immature immune systems and frequent exposure to contaminated environments, especially in school settings with insufficient sanitary infrastructure (Fauziah et al., 2022).

Parasites are broadly classified as ectoparasites or endoparasites based on their location on or within the host. Further classification considers life cycle complexity and host specificity, with some parasites exhibiting direct life cycles and others requiring multiple hosts (Loddo et al., 2018; Veiga et al., 2019). Among intestinal parasites, Ascaris lumbricoides, Trichuris trichiura, and hookworms are the most prevalent and clinically significant in Nigeria, causing malnutrition, anaemia, impaired cognitive

development, and decreased school performance among children (Adedokun et al., 2018; Adeniran et al., 2019).

Several regional studies across Nigeria have reported high prevalence rates of intestinal parasitic infections among school-aged children, ranging from 52% to 65% (Oyeyemi et al., 2020; Adeleke et al., 2019; Bala et al., 2018). In Edo State, prevalence rates have varied between 25.2% and 77.3%, depending on location and population studied (Omuemu et al., 2010; Egharevba & Okodua, 2013; Ekeh & Okafor, 2012). Notably, a study in Ovia North-East LGA reported a prevalence of 58.7%, with the highest burden among children aged 5–10 years (Egharevba & Okodua, 2013).

Ascaris lumbricoides, the most widespread soil-transmitted helminth, inhabits the human small intestine and causes ascariasis. Transmission occurs via ingestion of eggs in contaminated food or soil. The parasite's life cycle includes a complex pulmonary migration phase before settling in the gut, where adult worms can grow up to 30 cm and lay thousands of eggs daily (Jourdan et al., 2018; Hajare et al., 2022). Other major parasites, such as Trichuris trichiura and hookworms, contribute to chronic gastrointestinal symptoms, anaemia, growth retardation, and impaired cognitive development (Caldrer et al., 2022; Clements & Alene, 2022).

Protozoan parasites also play significant roles in intestinal morbidity. Giardia lamblia and Entamoeba histolytica cause giardiasis and amoebiasis respectively, both of which are associated with diarrhoea, malabsorption, and failure to thrive in children (Adam, 2021; Guillén, 2023). Strongyloides stercoralis, a unique nematode with autoinfective capacity, can persist chronically and cause life-threatening disseminated infections in immunocompromised hosts (Page et al., 2018; Yeh et al., 2023).

Given the persistent burden of intestinal parasitic infections in Nigeria, particularly among children, there is an urgent need to understand their epidemiology at the local level. This study aims to determine the prevalence and distribution of intestinal parasites among primary school children in the Southern Senatorial District of Edo State, Nigeria, to inform targeted control strategies and health interventions.

Materials and Methods

A descriptive cross-sectional study was conducted to determine the prevalence and molecular characterization of Ascaris lumbricoides among primary school children in the Southern Senatorial District of Edo State, Nigeria.

Study Location

Edo South Senatorial District is one of the three senatorial districts in Edo State, located in the South-South geopolitical zone of Nigeria. It comprises seven local government areas (LGAs): Oredo, Egor, Ikpoba-Okha, Uhunmwonde, Ovia South-West, Ovia North-East, and Orhionmwon. The region is predominantly urban and semi-urban, with a mix of public and private primary schools. The area has a tropical climate, characterized by distinct wet and dry seasons, and faces ongoing public health challenges related to water, sanitation, and hygiene infrastructure, making it a suitable site for parasitological studies.

Selection Criteria

Inclusion Criteria:

1. Pupils aged 5–10 years.
2. Enrollment in primary schools within the seven LGAs of Edo South.
3. Written informed consent from parents/guardians and assent from pupils.
4. No history of anthelmintic treatment within three months.
5. Residency in the selected LGAs for at least six months.

Exclusion Criteria:

1. Pupils outside the 5–10-year age range.
2. Pupils not enrolled in primary schools in the study area.
3. Lack of consent/assent.
4. Recent anthelmintic drug use.
5. Chronic illnesses or severe diarrhoea at the time of sample collection.
6. Less than six months' residence in the study area.

Study Population

A total of 633 pupils (392 females and 241 males), aged 5–10 years, from selected public primary schools across the seven LGAs of Edo South were enrolled.

Sampling Technique

A two-stage sampling method was employed:

Stage 1: School Selection

Primary schools were selected using simple random sampling from a list provided by the Edo State Ministry of Education. Schools were chosen across the seven LGAs, ensuring geographic representation.

Stage 2: Pupil Selection

Within selected schools, pupils were stratified by age (5–6, 7–8, 9–10 years) and gender. Proportional allocation was used to determine the number of participants per stratum. Systematic random sampling was then used within each stratum to select participants from school registers.

Method of Data Collection

Structured questionnaires were administered to pupils under the supervision of their teachers or guardians to gather sociodemographic and hygiene-related information.

Sample Collection

Labeled, sterile stool containers were distributed to participants, who were instructed to provide about 3 grams of fresh stool. Samples were transported within one hour to the laboratory and processed immediately.

Sample Examination

Macroscopic Examination

Stool samples were assessed for color, consistency, presence of blood or mucus, and visible adult parasites.

Microscopic Examination

Three techniques were employed:

a) Saline Smear: A saline suspension of stool was prepared on a slide and examined under ×10 and ×40 objectives for ova and larvae (Cheesbrough, 2022).

b) Iodine Smear: Lugol's iodine was used to stain stool smears, enhancing visualization of cysts and ova (Cheesbrough, 2022).

c) Formol-Ether Concentration: Approximately 1 g of stool was emulsified in formol water, sieved, and centrifuged with ether to concentrate ova. Sediments were examined microscopically for parasites (Cheesbrough, 2022).

RESULTS

Prevalence of Intestinal Parasites

A total of 633 primary school children were included in the study. The age distribution showed that 23.5% of the respondents were between 5 and 7 years, 49.4% were between 8 and 9 years, and 27.0% were between 9 and 10 years. In terms of gender, 38.1% were male and 61.9% were female. Regarding religious affiliation, the majority of respondents (99.4%) identified as Christians, while only 0.6% identified as Muslims. Participants were drawn from seven Local Government Areas within the Edo South Senatorial District: Egor (19.3%), Oredo (13.7%), Ikpoba Okha (21.3%), Uhunmwonde (7.9%), Ovia South West (12.0%), Ovia North East (16.6%), and Orhionmwon (9.2%) (Table 4.1). Ugbowo Primary School recorded the highest number of respondents (approximately 47), followed by Ozolua Primary School (around 37) and Ogida MPS (approximately 33). These top three schools contributed a substantial proportion of the total respondents. Following closely are Uvbi PS, Egba, Obazagbon, Ekhiri, and Eresoyen, each contributing similar numbers (around 30 respondents each). Other schools such as Lucy, Idogbo, Uwa PS, and Azogba show slightly lower but comparable numbers, ranging from approximately 25 to 30 respondents. The majority of respondents, 384 (60.7%), reported using tap water as their main source of drinking water. Well water was utilized by 248 (39.2%) of the respondents, while river water was the least reported source, accounting for only 1 (0.2%) of the total respondents (Figure 4.1).

Out of the 633 primary school children examined, 145 (22.9%) tested positive for parasitic infections, while 488 (77.1%) were negative (Figure 4.2). Among the 145 infected primary school children, Ascaris lumbricoides was the most frequently identified parasite, accounting for 55.2% of infections. Hookworm was detected in 21.4% of the cases, while Taenia species were identified in 15.9% of the infected children. Entamoeba histolytica accounted for 6.9% of infections, whereas Strongyloides stercoralis was the least prevalent parasite, detected in only 0.7% of cases (Table 4.2).

Table 1. **Sociodemographic Characteristics and Risk Factors (Water Source) of Respondents**

Variable	Frequency	Percentage
Age (Years)		
5-7	149	23.5
8-9	313	49.4
9-10	171	27.0
Total	**633**	**100.0**
Gender		
Male	241	38.1
Female	392	61.9
Total	**633**	**100.0**
Religion		
Christianity	629	99.4
Islam	4	0.6
Total	**633**	**100.0**
Local Government Area in the Southern Senatorial District of Edo State		
Egor	122	19.3
Oredo	87	13.7
Ikpoba Okha	135	21.3
Uhunmwonde	50	7.9
Ovia South West	76	12.0
Ovia North East	105	16.6
Orhionmwon	58	9.2
Total	**633**	**100.0**
Water Source		
Tap	384	60.7
Well	248	39.2
River	1	0.2
Total	**633**	**100.0**

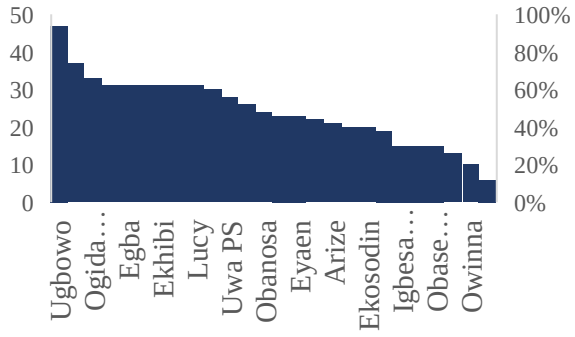

Figure .1. Distribution of Respondents from different primary schools across the southern senatorial district of Edo State.

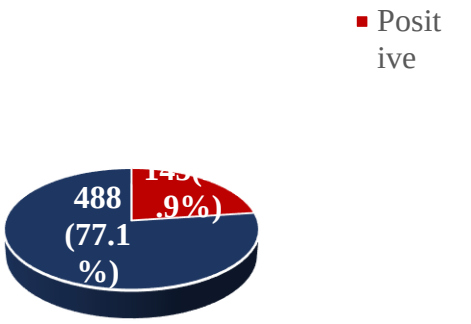

Figure .2. Prevalence of Intestinal parasites among primary school children in the Southern Senatorial zone of Edo State.

Table.1. **Distribution of Intestinal Parasites among Infected Primary School Children in the Southern Senatorial Zone of Edo state**

Parasites	Frequency	Percentage
Ascaris species.	80	55.2
Ancylostoma spp.s	31	21.4
Taenia species	23	15.9
Entamoeba histolytica	10	6.9
Strongyloides stercoralis	1	0.7
Total	**145**	**100.0**

Table.3 presents the prevalence of parasitic infection among primary school children in Edo South Senatorial District in relation to their sociodemographic characteristics. Age was not significantly associated with parasitic infection (χ^2 = 3.396, p = 0.183). Although children aged 9–10 years exhibited the highest prevalence (26.3%), followed closely by those aged 5–7 years (25.5%), and the lowest prevalence was among those aged 8–9 years (19.8%), the differences were not statistically significant. Gender was also not significantly associated with parasitic infection (χ^2 = 0.546, p = 0.460). Males recorded a slightly higher infection rate (24.5%) compared to females (21.9%). Religion did not show a significant association with parasitic infection (χ^2 = 0.010, p = 0.920). Among Christians, 22.9% were infected, while 25.0% of Muslim respondents were infected. Local Government Area (LGA) of residence demonstrated a statistically significant association with parasitic infection (χ^2 = 56.852, p = 0.0001). Infection rates varied markedly across the LGAs, with the highest prevalence observed in Ikpoba Okha (25.5%), Orhionmwon (20.7%), and Oredo (16.6%), whereas Ovia South West (8.3%), Egor (9.0%), and Ovia North East (9.0%) recorded lower infection rates.

Table 4 shows the prevalence of parasitic infection among primary school children in the Southern Senatorial District of Edo State in relation to their water source. A statistically significant association was observed between water source and parasitic infection (χ^2 = 48.084, p = 0.0001). Children who sourced their drinking water from wells exhibited the highest prevalence of infection (36.7%), compared to those who used tap water (13.8%). The single respondent who relied on river water was also infected (0.7%).

Table 2.Relationship Between Socio-demographic Factors and **Prevalence of Intestinal Parasite among Primary school Children in the Southern Senatorial District of Edo State.**

Socio-demographics	Number Examined (%)	Number Infected (%)	χ2	p- value
Age (Years)				
5-7	149 (23.5)	38 (25.5)	3.396	0.183
8-9	313 (49.4)	62 (19.8)		
9-10	171 (27.0)	45 (26.3)		
Gender				
Male	241 (38.1)	59 (24.5)	0.546	0.460
Female	392 (61.9)	86 (21.9)		
Religion				
Christianity	629 (99.4)	144 (22.9)	0.010	0.920
Islam	4 (0.6)	1 (25.0)		
Local Government Area in the Southern Senatorial District of Edo State				
Egor	122 (19.3)	11 (9.0)	56.852	**0.0001***
Oredo	87 (13.7)	24 (16.6)		
Ikpoba Okha	135 (21.3)	37 (25.5)		
Uhunmwonde	50 (7.9)	18 (12.4)		
Ovia South West	76 (12.0)	12 (8.3)		
Ovia North East	105 (16.6)	13 (9.0)		
Orhionmwon	58 (9.2)	30 (20.7)		

* Represents statistical significance at $p<0.05$

Table 3.Relationship Between Risk Factors (Water Source) and **Prevalence of Intestinal Parasite among Primary school Children in the Southern Senatorial District of Edo State.**

Risk Factor	Number Examined (%)	Number Infected (%)	χ2	p- value
Water source				
Tap	384 (60.7)	53 (13.8)	48.084	**0.0001***
Well	248 (39.2)	91 (36.7)		
River	1 (0.2)	1 (0.7)		
Total	**633 (100)**	**145 (100)**		

* Represents statistical significance at $p<0.05$

Discussion

The findings of this study provide critical insights into the epidemiology and molecular characteristics of intestinal parasitic infections (IPIs) among primary school children in the Southern Senatorial District of Edo State, Nigeria. By integrating demographic, environmental, parasitological, and molecular data, this study offers a comprehensive understanding of the burden, distribution, and diversity of intestinal parasites in the region. These insights are crucial for informing public health strategies and guiding targeted interventions aimed at reducing the transmission of IPIs among vulnerable pediatric populations.

A significant gender disparity was observed among the study participants, with female pupils outnumbering males. This overrepresentation may reflect better

school enrollment and retention rates for girls in the study area, possibly due to gender-focused educational initiatives or sociocultural shifts that encourage female education. While some studies corroborate this trend (e.g., Pal, 2010; Kazeem et al., 2010), others report near-equal gender representation in school-based studies (Gowon et al., 2018; Dangana et al., 2011). In contrast, in rural communities, boys are sometimes more engaged in labor-intensive roles or agricultural activities, resulting in lower classroom attendance and thereby impacting gender distribution in school-based surveys.

Local Government Area (LGA) representation in the study showed that pupils from Egor, Ikpoba-Okha, and Ovia North-East LGAs were most represented, while Uhunmwonde had the least. This disparity can be attributed to differences in population density, number of public schools, and ease of access to educational infrastructure. Urban LGAs generally benefit from better access to education, healthcare, and sanitation, which are essential factors influencing both school attendance and disease transmission. Conversely, rural LGAs like Uhunmwonde may face infrastructural challenges, longer travel distances to school, and lower socioeconomic indices, all of which impact school participation and public health outcomes.

The age distribution of the children (predominantly 7 to 9 years) aligns with standard primary school enrollment patterns in Nigeria, corroborating similar observations in previous studies (Gowon et al., 2018; Dangana et al., 2011). This age group is particularly vulnerable to parasitic infections due to underdeveloped hygiene practices, frequent exposure to contaminated environments, and behavioral tendencies such as playing barefoot or failing to wash hands after defecation.

One of the key environmental variables assessed in this study was the source of drinking water, which varied significantly among participants. Pupils reported accessing tap water, well water, and river water. The reliance on well and river water—particularly in rural or peri-urban LGAs—raises concerns, as these sources are more susceptible to contamination from open defecation, agricultural runoff, and unprotected storage. The use of river water is especially alarming, as it is strongly linked with waterborne diseases, including protozoal and helminthic infections (Dos Santos et al., 2017; Ntouda et al., 2013). Tap water, although perceived as safer, may still pose a risk in areas where municipal supply is intermittent or poorly maintained. Such variability in water access plays a central role in the transmission dynamics of intestinal parasites.

The religious homogeneity of the participants—predominantly Christian—reflects the sociocultural context of the Southern Senatorial District of Edo State, where Christianity is the dominant faith. This demographic finding is consistent with that of Asaolu et al. (2002) and, while not directly influencing parasitic infections, may have indirect implications through faith-based hygiene education and health outreach programs.

From a parasitological standpoint, the study recorded a prevalence of 22.9% (145 out of 633 pupils), indicating a moderate but concerning burden of intestinal parasitic infections in the population. While lower than rates reported in some regions—such as South Africa (64.8%) by Nxasana et al. (2013), Ethiopia (41.4%) by Abossie and Seid (2014), or Laos (61.9%) by Rim et al. (2003)—this prevalence still represents a significant public health issue. Conversely, it is higher than that recorded in Tehran, Iran (18.4%) by Nematian et al. (2004), emphasizing the regional variation in parasite burden influenced by hygiene infrastructure, climate, socioeconomic status, and health literacy.

Within Nigeria, the 22.9% prevalence observed aligns closely with the 23.95% reported by Gbonhinbor et al. (2022) in Bayelsa State but contrasts sharply with higher rates in Imo (48.7%) and Delta (50.0%) states (Eboh et al., 2022; Ugochi et al., 2015). These discrepancies may stem from environmental, behavioral, or methodological differences across regions, including sampling techniques, diagnostic sensitivity, and seasonal timing of the studies.

The spectrum of intestinal parasites identified included Ascaris lumbricoides, Ancylostoma spp., Taenia species, Entamoeba histolytica, and Strongyloides stercoralis. Ascaris lumbricoides was the most prevalent, consistent with earlier findings across Nigeria and other sub-Saharan countries (Rim et al., 2003; Ugochi et al., 2015; Gbonhinbor et al., 2022). Its dominance is often linked to persistent soil contamination, poor sanitation practices, and the parasite's resilience in varied environmental conditions. The transmission of Ascaris is typically fecal-oral, facilitated by inadequate hand hygiene and open defecation, especially in communities lacking latrine facilities.

Hookworm infections were the second most common. These parasites are transmitted via skin penetration, particularly through walking barefoot on contaminated soil—a common behavior among children. The warm, humid climate of southern Nigeria further supports larval development in the soil, enhancing transmission potential (Narahari et al., 2016).

Taenia species, though less prevalent, highlight the continued risk of taeniasis due to poor meat inspection practices and the consumption of undercooked pork or beef. The presence of Entamoeba histolytica—a waterborne protozoan—confirms that protozoal infections persist in some communities, albeit at a lower rate, likely due to partial improvements in water safety. Strongyloides stercoralis was the least detected, consistent with other studies reporting its lower prevalence in school-age children, possibly due to its more complex lifecycle and requirement for specific ecological conditions (Alegría et al., 2017; Salim et al., 2014).

Among the sociodemographic and environmental variables analyzed, the LGA of residence emerged as a significant determinant of infection risk. Pupils from Ikpoba-Okha, Orhionmwon, and Oredo recorded the highest infection rates. These variations may reflect differences in sanitation infrastructure, access to clean water, hygiene education, and community health practices across LGAs (Onabolu et al., 2011). This observation is in line with Gbonhinbor et al. (2022), who found significant spatial heterogeneity in parasitic infection prevalence across nine communities.

Other variables—such as age, gender, and religion—did not show statistically significant associations with parasitic infections, although marginal differences were noted. This may indicate a relatively uniform exposure to risk factors across groups or the possibility that environmental variables exert a stronger influence than individual demographics—a finding also supported by Ugochi et al. (2015).

Water source, on the other hand, showed a significant association with infection prevalence. Pupils using well water had a notably higher risk of infection than those with access to tap water, underscoring the need for improved water quality management. These findings align with studies by Grimes et al. (2015) and Fuhrimann et al. (2016), which emphasized the role of unsafe water in sustaining parasitic transmission cycles.

In conclusion, the results of this study underscore the continuing burden of intestinal parasitic infections among schoolchildren in Edo South, with Ascaris lumbricoides as the predominant species. The findings emphasize the urgent need for integrated control strategies, including mass deworming programs, improved water and sanitation infrastructure, health education, and periodic surveillance incorporating both microscopy and molecular diagnostics. Future research should also focus on genotype-specific behavior of Ascaris and other parasites to better understand patterns of resistance, reinfection, and pathogenicity.

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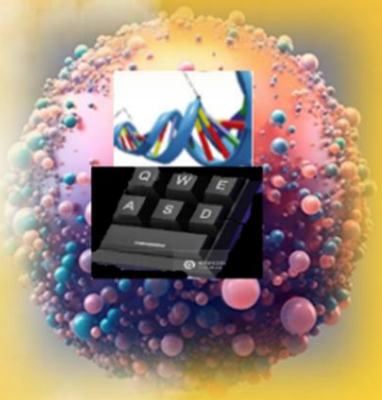

IUO_Journal of Natural and Applied Sciences Research (JNASR)
May 2025
ISSN: (in process)
Volume 1, Issue 1
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Edo State of Nigeria
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