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Review Article **Open Access**

A review of the epidemiology, diagnosis, treatment, vaccines and economic impact of human monkeypox (Mpox) outbreaks

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Abstract:

The current monkeypox outbreak is a public health emergency of international concern and is coming in the wake of the SARS-CoV-2 pandemic. Human monkeypox is a viral zoonotic infection caused by monkeypox virus, an enveloped double-stranded DNA virus of the genus *Orthopoxvirus* and family *Poxviridae* that also contain smallpox, cowpox, Orf, and vaccinia viruses. Online databases including PubMed, Google Scholar and Web of Science were searched to obtain relevant publications on the epidemiology, treatment, vaccines and the economic impacts of the current monkeypox (Mpox) outbreak.

Keywords: monkeypox, epidemiology, vaccines, treatment, economic impact

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Un examen de l'épidémiologie, du diagnostic, du traitement, des vaccins et des impact économiques des épidémies de monkeypox (Mpox) humain

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Résumé:

L'épidémie actuelle de monkeypox est une urgence de santé publique de portée internationale et survient dans le sillage de la pandémie de SRAS-CoV-2. Le monkeypox humain est une infection zoonotique virale causée par le virus du monkeypox, un virus à ADN double brin enveloppé du genre *Orthopoxvirus* et de la famille des *Poxviridae* qui contient également les virus de la variole, du cowpox, de l'Orf et de la vaccine. Des bases de données en ligne, notamment PubMed, Google Scholar et Web of Science, ont été consultées pour obtenir des publications pertinentes sur l'épidémiologie, le traitement, les vaccins et les impacts économiques de l'épidémie actuelle de monkeypox (Mpox).

Mots clés: monkeypox, épidémiologie, vaccins, traitement, impact économique

Introduction:

Human monkeypox is a viral zoonotic infection caused by monkeypox virus, an enveloped double-stranded DNA virus of the genus *Orthopoxvirus* belonging to the family *Poxviridae* which contains smallpox, cowpox, Orf, and vaccinia viruses (1,2). The virus is made

up of two genetic clades; the Central African (Congo Basin) clade, which is now known as clade 1 and more transmissible and causes more severe disease, and the West African clade (clade 2), which is thought to cause mild disease (1,3).

With the recent increase in monkeypox cases, changes in the geographic location of cases, with majority of infections occurring in regions that have not previously reported cases, and changes in the demography of infected persons, it has become imperative to review the epidemiology, diagnosis, treatment modalities, current vaccines available for prevention and the economic impact of monkeypox outbreak. This will enable researchers to understand the disease trends, shifts in geographical spread and changes in the epidemiological characteristics as a result of evolution of monkeypox virus and adaptation to the human immunity, thereby aiding the development of innovative surveillance systems, diagnostic techniques, treatment modalities and preventive measures including vaccines that will enable prevention and management of future outbreaks. This knowledge will also guide policy makers in making well informed decisions that will positively impact the economy.

The objective of this review is to describe the epidemiology of human monkeypox, diagnosis, available treatment options, current vaccines approved for use, and the economic impacts of the current human monkeypox outbreak.

Methodology and Results:

Online electronic databases including PubMed, Google Scholar and Web of Science were searched for primary source articles including original reports, case series studies, case reports, seroprevalence studies and epidemiological reports on human monkeypox. Secondary search was also conducted using references of the primary articles reviewed. Inclusion criteria for selecting studies for the review were studies that provided information on human monkeypox history, diagnosis, clinical features, treatment, vaccination and economic impacts. Search words used include 'human monkeypox' OR 'monkeypox' AND 'monkeypox origin' OR 'history' AND 'monkeypox epidemiology' AND 'monkeypox treatment' AND/OR 'management' AND 'monkeypox vaccines', AND 'monkeypox economic impact'. Studies on reviews and systematic reviews of monkeypox were excluded.

Using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guide, initial search produced 2310 articles on Google Scholar, 1097 articles from PubMed and 2593 from Web of Science. Following de-duplication, 1090 article titles and abstracts were screened, followed by assessment of 207 full text publications to determine eligibility, and 158 publications were further excluded, leaving 49 eligible articles. A further 28 eligible articles were obtained from secondary searches, giving a total of 77 eligible publications for the review (Fig 1).

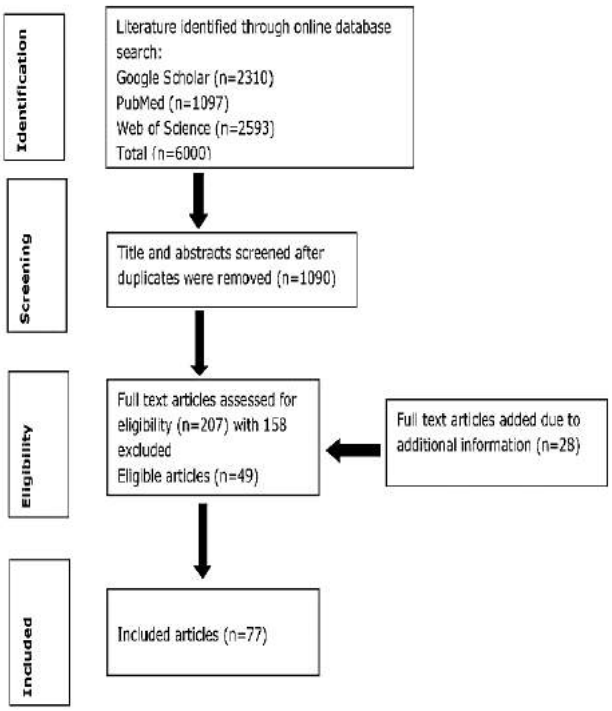

Fig. 1: Process of selection of publications (PRISMA guide) used for the review

Discussion:

Historical background:

Monkeypox was first identified in 1958 in captive monkeys that developed vesicular disease while being transported from Africa to Copenhagen, Denmark for research (4). Although this discovery earned the disease its name, the largest animal reservoirs of the virus are rodents including squirrels and giant pouched rats (4). The World Health Organization (WHO) recently renamed monkeypox as 'Mpox' following reports from countries and individuals of racist and stigmatized language being used in various settings and communities (5). The natural host reservoir is still unknown but the animal hosts are varied and include squirrels, Gambian pouched rats, dormice and non-human primates (1,6-10).

There is plethora of information on monkeypox that remains unknown, which include its mode of transmission from animal to human host, pathogen-host associations, factors that enable the virus to persist in nature, environmental factors that influence the virus

geographical shift are research questions to be answered to help in controlling and preventing monkeypox virus spread. The drivers of transmission to humans are aerosol transmission, documented in animal transmission (11,12) as well as direct and indirect contact with infected live or dead animals (1,13-15). Human-to-human transmission occurs through close contact with respiratory secretions, skin lesions of infected persons and contaminated objects (1,15), placental transmission and close contact during and after birth (1,16). Sexual transmission has not been confirmed and requires further investigation.

Clinical monkeypox virus infections:

Human monkeypox was first diagnosed in the Democratic Republic of the Congo (DRC) in a 9-month-old boy following the cessation of smallpox vaccination, after smallpox eradication in the late 1970s (1,17). The clinical manifestations of human monkeypox are indistinguishable from those of smallpox, but severity and mortality are much lower with monkeypox. The disease is characterized by fever, headache, myalgia, tiredness and marked lymphadenopathy involving the cervical and inguinal lymph nodes, and differs from smallpox and chickenpox in this respect.

The symptoms of monkeypox are milder because of previous immunization with vaccinia virus vaccine during the smallpox eradication programme, at which time individuals developed cross immunity against monkeypox (18). Monkeypox is endemic in Central and West Africa with more cases being seen in rural tropical rainforest regions, where contact with animal hosts is more frequent, although there has been increasing spread to urban areas with urbanization encroaching into the rural areas (7,19). In addition, political instability and insecurity in some of these endemic areas leads to displacement of large numbers of people who then live close to these animal hosts. The practice of many inhabitants hunting wild animals for food, is also a predisposing factor. Other African countries that have reported cases of human monkeypox include Benin, Cameroon, Central African Rep ublic, Côte d'Ivoire, Gabon, Liberia, Nigeria, the Republic of Congo, Sierra Leone and South Sudan.

Epidemiology of monkeypox

There have been several outbreaks of human monkeypox after it was first identified in 1970. The 1970-79 outbreaks affected individuals in DRC, Sierra Leone, Nigeria, Liberia and Ivory Coast resulting in 47 cases, with the majority (n=38) from Zaire and the DRC (20). Although cases are seen all year round, the outbreak occurred most often in the dry season and clustering was noted within families, localities and countries (20,21). Children under 10 years of age (range of 7 months to 35 years) were most affected. The sex ratio was similar with 26 males and 21 females affected. Among females, adult females were more at risk. Incubation period was about 12 days (approximately 7-14 days) (20,22). The onset of symptoms was within 24 hours to five days while onset of rash was between 9 to 17 days. Symptoms ranged from mild to severe and mostly observed among unvaccinated individuals. Mode of transmission was either directly from contact with animals carrying the virus or secondarily through person-to-person transmission (usually milder and atypical) (20).

The DRC had another outbreak between 1981 to 1986 with 338 cases (67% confirmed by virus culture), 182 male and age range of 3 months to 69 years (23). Majority of the cases (86%) were children below 10 years of age and 13% of all cases had vaccination scar. Among cases identified, 72.5% had primary infection and 93 patients were considered to be secondary cases. Attack rates (AR) among unvaccinated persons was 7.47% while AR among unvaccinated individuals living in the same household with cases was 9.3% (7 times higher than AR for vaccinated household contacts) (23). Case fatality rate (CFR) was below 10% which was significantly lower than for smallpox. It was also noted that transmission beyond secondary infection was rare and it was established that repeated reintroduction of the virus was necessary to sustain the disease outbreak (24). In 1984, an outbreak was also reported in the Central African Republic. Six cases were identified among unvaccinated individuals in a pygmy community (25).

A third outbreak occurred in the DRC between 1996-1997. In 1996, Katako-Kombe health zone, Kasai Oriental, DRC was considered the epicenter of the outbreak with 71 cases identified and mode of transmission was predominantly person - to - person (7). From February 1996 to February 1997, 88 clinical cases were identified in nine of 12 villages (AR of 22 per 1000), of which 50 were males. The median age of onset was 10 years (range from 1 month to 62 years). In keeping with its occurrence mainly in the dry season, cases increased from February 1996, peaked in August 1996 and subsequently declined. This trend was the same in 1997 when there was a resurgence in February 1997 with AR being similar in males and females and ranging from 0-105 per 1000 in Akungula (7). By age, children less than 15 years of age were mostly affected (AR of 146 per 1000). As of October 1997, 419 cases were identified; 344 in Katako-Kombe health zone (AR of 1.1 per 1000)

and 75 in Lodja health zone (AR of 0.3 per 1000). Five deaths were reported among children 4 to 8 years of age in Katako-Kombe (1.5% case fatality ratio). Majority of the cases were in individuals less than 16 years and 78% of cases were secondary infections. Among the cases, only 6% had previously received vaccinia vaccine putting the secondary AR at 8% (7). With each outbreak, there have been an increasing number of cases. This has been attributed to the waning cross-immunity among those vaccinated with the smallpox virus, increasing number of young unvaccinated individuals and increasing human contact with small animals that could serve as hosts for monkeypox virus.

In 2003, 11 cases were detected in the Republic of Congo among children below 18 years of age, which resulted in one death and one individual with profound sequelae. It was during this outbreak that sustained person-to-person transmission was initially observed (26). The DRC also experienced an outbreak during this period (2001-2004) with over 2000 suspected cases reported and clinical samples obtained from 136 individuals as a result of the civil unrest which hindered surveillance activities. Fifty-one cases were confirmed from 136 suspected case samples collected, male-to-female ratio was approximately equal and age ranged from 2 months to 54 years with majority of cases occurring in children below 10 years (27). Human monkeypox was also first reported in the United States in 2003 (28). A total of 50 cases were identified in Wisconsin, Illinois, Indiana and New Jersey. Fifty-five percent were females, age ranged between 1 - 59 years (median - 28 years) with most being primary infections from contact with infected prairie dogs. Diagnosis was through culture or DNA amplification of skin lesion samples and antibody neutralization assay. There were no deaths reported during this outbreak (28).

Sudan experienced an outbreak in 2005 with 10 confirmed cases and 9 probable cases (29,30). Surveillance data from the DRC between 2005 to 2007 revealed 760 laboratory-confirmed monkeypox cases with preponderance for people living close to forested areas, males, below 15 years of age and unvaccinated (31). Between 2000 and 2009, the Republic of Congo reported 27 cases while the DRC reported 10,027 suspected cases (32, 33). From 2010 to 2019, 2 cases were reported in Sierra Leone, 6 in Liberia, 3 in Cameroon, 24 in Congo, 61 in Central African Republic, 181 in Nigeria and over 8,700 suspected cases in the DRC (33-46).

The outbreak in Nigeria was as a result of the West African clade in 2017-2018 with 122 confirmed cases across 17 States (45). Male individuals were mostly affected (n=84) and the age range of infected persons was between 2 days to 50 years, with median age being 29 years. Seven deaths were reported with a case fatality rate of 6%. Transmission was through primary zoonosis and secondary person-to-person. All the cases had rash and other common symptoms including lymphadenopathy, fever, itching and headache. By January 2019, there were 311 suspected cases across 26 States, of which 132 cases were confirmed. Most cases were between 21 and 40 years (median age of 31 years). Seven deaths were also reported (46). In 2018, three cases were identified in the United Kingdom, two travelled from Nigeria and one was a health worker (46,48). In October of the same year, another case from Nigeria was identified in Israel. In 2019, a case was imported to Singapore while in 2021, another was detected in the USA (49,50,51). In the UK, cases were subsequently detected in 2019, 2021 and 2022 (52,53). The 2021 outbreak in Nigeria was not widespread and the reported number of cases were 32 with no deaths (54).

In 2022, monkeypox became an infection with global impact as it became the largest monkeypox outbreak observed in many countries where it had not previously been seen and declared a public health emergency of international concern (1,55-61). As of September 23 2022, about 106 countries have reported cases, with over 65000 cases globally and majority of cases (approximately 64,800 in 99 locations) have been reported in countries outside Africa (62). The highest numbers have been seen in North America and Europe. Deaths have been equally split between countries where monkeypox is endemic (10 deaths in 3 locations) and locations that have not previously reported cases (10 deaths in 8 locations) (62). Majority (~99%) of cases have been among males, with men who have sex with men (MSM) being disproportionately affected (60,61,66). This has raised the question as to whether it should be termed a sexually transmitted infection (STI) (63-65). Although all age groups are affected, adult males between 26-40 years are worse hit. At the beginning of the outbreak, majority of cases (67%) were seen among Whites but as the outbreak progressed, cases increased among individuals in the minority ethnicity (Hispanics -27% and African Americans-41%) while the number of cases among White reduced (26%) (66). These demographics in the UK were similar (60,61).

While monkeypox is not an STI, it spreads rapidly through sexual and close contact. Person-to-person transmission through close contact with lesions, respiratory droplets, body fluids and contaminated materials are

the major drivers of this outbreak (60,61,66) Other risk factors include eating inadequately cooked infected meat and animal products. Incubation period is from five to 21 days and there are two phases of illness. Invasive (prodrome) phase which is 0-5 days where infected persons experience fever, lymph node enlargement, malaise, headache, itching, chills and myalgia. Lymphadenopathy is a distinguishing sign of monkeypox which differentiates it from other infections with similar signs and symptoms. Skin eruption phase lasts 1-3 days after fever onset and is associated with rash that is predisposed to the face and extremities; 95% of cases have reported facial rash and 75% have experienced rash on the palms of hands and soles of feet (61,62,66). Without treatment, the symptoms usually clear within 2-4 weeks and mortality is between 3-6%, pre dominantly among children and immunocompromised persons.

Diagnosis of monkeypox:

Confirmatory diagnosis of monkeypox has been through polymerase chain reaction (PCR) testing of samples taken from skin lesions because of its high sensitivity and accuracy (60,67). Genomic sequencing is also used as a diagnostic tool (3,68). Serological and antigen detection are not recommended due to cross-reactivity between the different ortho poxviruses.

Treatment of monkeypox:

Treatment of human Monkeypox is mainly symptomatic as there are no drugs developed specifically for monkeypox treatment. Due to the cross sensitivity among the orthopoxviruses, an antiviral drug, tecovirimat, developed for management of smallpox is used in a small percentage of cases, specifically in individuals who have or are prone to developing severe form of the disease including immunocompromised persons and individuals with skin conditions such as eczema (69,70). Cidofovir is also used for treatment in some infected persons (60).

Targeting viral protein p37, a highly conserved domain found in all orthopoxviruses, tecovirimat is able to inhibit the formation of the envelopes of monkeypox virus. It is normally given twice daily as oral capsules for fourteen days. The US FDA has recently approved the intravenous form of the drug (on May 19, 2022), which has been found effective in treating primates (71). The 2022 global monkeypox outbreak is different from previous outbreaks in the mode of transmission, at risk groups and geographic spread, which could well be as a result of viral mutations and waning immunity. People engaging in same sex practices have shown higher incidence in Europe and North America (60,61,62)

Vaccines against monkeypox:

Infection by any of the orthopoxviruses can confer immunity against infection from other species in the family. This is beneficial as people who were immunized with the vaccinia virus vaccine during the smallpox eradication are protected from the smallpox, monkeypox and cowpox diseases. With the eradication of smallpox, immunity has waned leading to resurgence of infections by orthopoxviruses especially among unvaccinated individuals.

There are two vaccines currently approved for prevention of monkeypox. The first is Jynneos (Imvamune or Imvanex), a non-replicating live virus vaccine which is being used to prevent monkeypox and smallpox disease by administration four days before exposure is the primary vaccine approved for use (72). Two doses taken 28 days apart are required to provide optimal immune response. Immunity is conferred 14 days after the second dose. It is approved for use in all ages. Although Jynneos has been shown to be effective in animal and clinical studies, more information is being gathered on its effectiveness, side effects and long-term protective effects. It is also recommended for use in addition to other preventive measures in place. Most common side effects reported include redness, swelling and itching at the injection site, headache, tiredness, muscle aches, chills and nausea (72).

The second vaccine, called ACAM2000 was developed to protect against smallpox but expected to be effective in preventing monkey pox. Therefore, it is approved as an alternative to Jynneos. ACAM2000 is a live vaccinia virus vaccine (73) and a single dose vaccine administered by giving multiple pricks to the upper arm with a special needle. Special care is needed for the lesion. Optimal immune response is expected 28 days after administration. ACAM2000 is contraindicated in persons with weakened immune system including people with HIV, persons on chemotherapy; pregnant and breastfeeding women; individuals with heart conditions and skin conditions such as psoriasis, eczema, dermatitis; infants below 12 months and individuals who will be unable to self-isolate from persons who have these conditions (73). Unlike Jynneos that can be administered with other vaccines, ACAM2000 cannot be administered with other live injectable vaccines and it is recommended that other vaccines be administered 4 weeks after receiving ACAM2000. Side effects are similar to Jynneos but in addition, a rash may develop and swollen glands may be observed. It is also

associated with more frequent side effects (73).

Other preventive measures recommended include avoiding close or skin-to-skin contact with people who have a rash that looks like monkeypox, avoid touching surfaces, objects and materials used by infected persons and frequent hand washing (74). For those at high risk of infection, limiting activities that increase chances of exposure, limiting number of sex partners and engaging in safer sexual practices (75). Pet animals can be infected and transmit monkeypox therefore, measures should be taken to prevent infection and spread of the virus (76). Preventive measures should also be taken in congregate settings to prevent spread of the virus and in a situation where a monkeypox case is detected, the staff, residents and local authorities should be notified, isolate infected individual and ensure other preventive measures are adhered to so as to avoid the spread of the virus (77).

Economic impacts of monkeypox outbreaks:

With the monkeypox outbreak being a public health emergency of international concern and coming in the wake of SARS-CoV-2 pandemic, the already stretched thin resources have to be further diversified to conduct robust surveillance, research, develop and deploy new vaccines, diagnostic tools and treatment drugs to prevent further spread of the disease. The vaccinia vaccine has proven to be effective but there are limited supplies and production of more vaccines have to be expedited and equitable distribution implemented.

In addition, although monkeypox is regarded as a public health emergency of international concern, it has not affected trade or travel unlike SARS-CoV-2 infection. With the emergence of monkeypox globally at a time the world is still grappling with SARS-COV-2 and with young individuals, that make up the majority of the workforce, being affected, this will negatively impact GDP of different countries in losses due to sick days taken off work, cost of treatment and strain on health care services and personnel.

Conclusion:

The current global pandemic of monkeypox reminds us that infectious diseases know no borders. Responses should therefore protect everyone, leaving no country behind. It is recommended that there should be equitable contributions from countries, with each country taking responsibility for providing solutions to the outbreaks at the national level. By applying resources responsibly, countries where these diseases are endemic can gradually move away from depending on richer countries, and effectively contribute to both national and global efforts to control pandemics.

Contributions of authors:

AB conceived the review idea, designed the outline, reviewed and edited the manuscript. MTO wrote the history, epidemiology, part of the clinical features and treatment, diagnosis, vaccines, economic impact and edited the manuscript. MN wrote part of the clinical features and treatment. BM reviewed and edited the manuscript.

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No conflict of interest is declared

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Mini-Review **Open Access**

The synergism of hepatitis B and aflatoxicosis in hepatocellular carcinoma development: A mini-review

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Abstract:

Hepatocellular carcinoma (HCC) is the twelfth most common cancer and the fifth leading cause of worldwide cancer-related death. Chronic hepatitis B infection, caused by the hepatitis B virus (HBV) and exposure to aflatoxins is fundamental in the formation of HCC in developing countries. This review of scientific publications aims to establish the detrimental effects of aflatoxin-contaminated foods and highlights the correlation between aflatoxin and hepatitis B viral-associated hepatocellular carcinoma. Research has shown a significant increase in the occurrence of HCC in HBV-infected individuals exposed to fungal toxins. HBV demonstrates the ability to integrate and bind to *p53* protein in the host DNA and propagate hepatocyte vulnerability through carcinogenic aflatoxin B_1 (AFB_1) damage. Although there has been clear evidence about the synergistic interaction of exposure to AFB_1 and HBV infection in the induction of HCC, other literature has shown otherwise, mainly because incomplete and vague findings and hypotheses were made in regions where AFB_1 and HBV pose a public health risk. Vaccination against hepatitis B and measures such as robust food safety systems to avoid hepatotoxicity and hepatocellular carcinogenesis induced by AFB_1 is the most effective methods in the prevention of HCC induced by HBV and AFB_1.

Keywords: aflatoxin B_1; hepatitis B; hepatocellular carcinoma; synergy

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La synergie de l'hépatite B et de l'aflatoxicose dans le développement du carcinome hépatocellulaire: une mini-revue

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Résumé:

Le carcinome hépatocellulaire (CHC) est le douzième cancer le plus fréquent et la cinquième cause de décès par cancer dans le monde. L'infection chronique par l'hépatite B, causée par le virus de l'hépatite B (VHB) et l'exposition aux aflatoxines est fondamentale dans la formation du CHC dans les pays en développement. Cette revue de publications scientifiques vise à établir les effets néfastes des aliments contaminés par l'aflatoxine et met en évidence la corrélation entre l'aflatoxine et le carcinome hépatocellulaire associé au virus de l'hépatite B. La recherche a montré une augmentation significative de la survenue de CHC chez les personnes infectées par le VHB exposées à des toxines fongiques. Le VHB démontre sa capacité à s'intégrer et à se lier à la protéine p53 dans l'ADN de l'hôte et à propager la vulnérabilité des hépatocytes par le biais de dommages cancérigènes à l'aflatoxine B_1 (AFB_1). Bien qu'il existe des preuves claires de l'interaction synergique de l'exposition à l'AFB_1 et à l'infection par le VHB dans l'induction du CHC,

d'autres publications ont montré le contraire, principalement parce que des conclusions et des hypothèses incomplètes et vagues ont été formulées dans des régions où l'AFB_1 et le VHB posent un risque pour la santé publique. La vaccination contre l'hépatite B et des mesures telles que des systèmes de sécurité alimentaire robustes pour éviter l'hépatotoxicité et la carcinogenèse hépatocellulaire induites par l'AFB_1 sont les méthodes les plus efficaces dans la prévention du CHC induit par le VHB et l'AFB_1.

Mots clés: aflatoxine B_1; hépatite B; carcinome hépatocellulaire; synergie

Introduction:

Hepatocellular carcinoma (HCC) is most frequent type of primary liver cancer in adults. Based on the number of cases reported each year, HCC (Plate 1) is the twelfth most common cancer and the fifth leading cause of all cancer-related deaths worldwide (1).

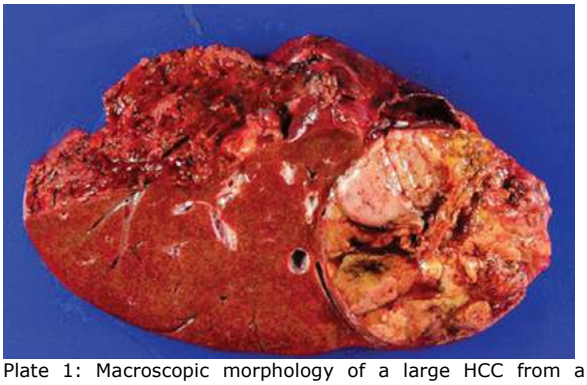
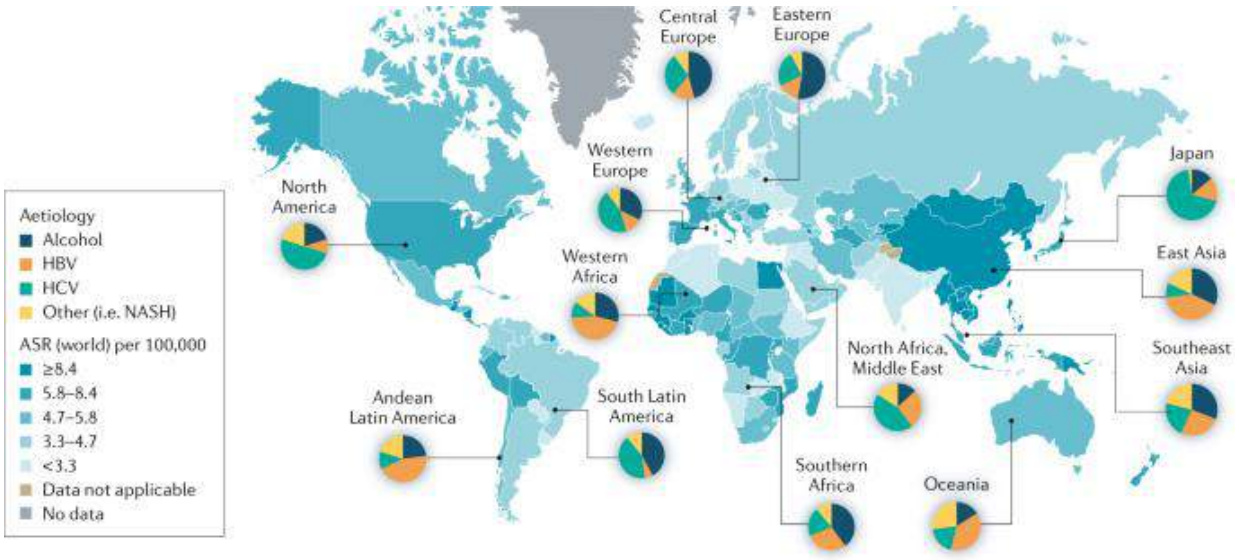


Plate 1: Macroscopic morphology of a large HCC from a patient with a co-infection of hepatitis B and aflatoxicosis (20)

HCC is an exigent disease that leads to the mortalities of greater than 600,000 people every year. In a recent study, 841,080 new cases of tumor were reported, accounting for up to 9.2% of all new cancers (3). Although HCC does not have a uniform geographical distribution as shown in Fig 1 (4), its overall incidence in developing countries at 513,000 new cases is five times that of the developed nations at 110,000 new cases (5). These rates have been apparent, especially in sub-Saharan Africa and the Asia Pacific (3). Chronic hepatitis B and C infection and aflatoxin (AFT) exposure are decisive in the manifestation of HCC in developing countries (6). However, excessive alcohol intake is also a major predisposing factor.

Viral hepatitis is the inflammation of the liver, which is caused by a viral infection (7). The infection may be manifested as acute (recent infection, relatively rapid onset) or chronic (persistent). Viral hepatitis may be caused by infection by one of the five currently known viruses,



This figure shows that the highest prevalence of HCC occurred in East Asia, while the highest worldwide was observed in Mongolia. The major aetiological factor in many parts Africa, South America and Asia (excluding Japan) is the Hepatitis B virus (HBV) while Hepatitis C virus (HCV) is the chief aetiological factor in Japan, North America and Western Europe. Alcohol intake is indicted in Central and Eastern Europe. Non-alcoholic steatohepatitis (NASH) is the major aetiological factor of the category tagged 'Other'; it is a fast-rising risk factor that could become the predominant aetiology of HCC. ASR is the age-standardized incidence rate.

Fig 1: Global map showing the prevalence and major aetiological factors of HCC (4)

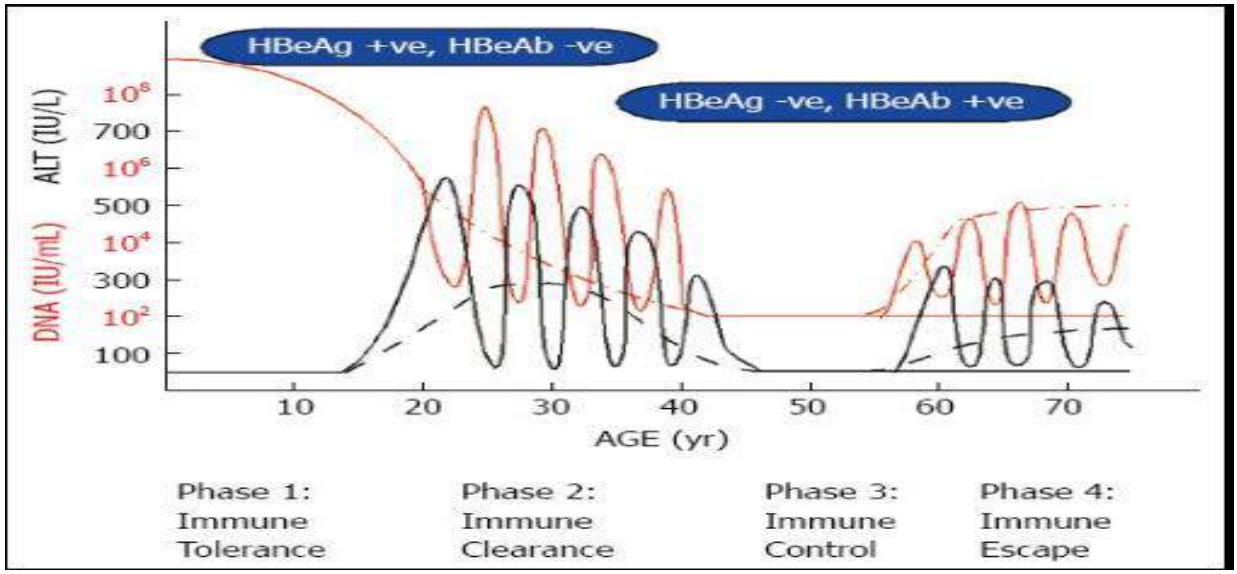


Fig 2: The natural history of CHB, demonstrating the interaction of serology, biochemistry and molecular virology (10)

which principally affect the liver; hepatitis A, B, C, D and E viruses (HAV, HBV, HCV, HDV and HEV) (8). Although there are certain similarities in the clinical manifestations caused by them, the hepatitis viruses differ widely in their morphology, genomic organization, taxonomic classification, and replication modes. The epidemiology of viral hepatitis infection is substantial. About 2 billion people exposed and 350 million people with chronic infection are at risk for developing liver disease and are responsible for nearly 1 million deaths annually (9).

Chronic HBV infection (Fig 2) associated with cirrhotic liver was categorized as the essential factor linked with the HCC development (10), which has been reported as one of the principal causes of death among men (11,12). In Africa, more than 65 million people are estimated to be chronic carriers of hepatitis B virus (10), which accounts for up to a quarter of the world's chronic HBV population and may be an understatement because of the lack of surveillance and underreporting in many African countries. Studies outside Africa cannot be extrapolated to Africa because HBV strains circulating in Africa differ from those found outside Africa and have unique characteristics.

Aflatoxins (AFTs) are extremely hazardous secondary metabolites obtained from polyketides associated with fungal species such as *Aspergillus flavus, A. parasiticus,* and *A. nomius* (13). These fungi typically affect cereal crops, including wheat, walnut, corn (Plate 2), cotton, peanuts, and tree nuts, during favorable temperatures and humidity (3). They can cause toxic damage to humans and animals, such as hepatotoxicity, teratogenicity, and immunotoxicity (14). AFTs are classified as group 1 carcinogenic agents to humans by the International Agency for Research on Cancer (IARC) (5). Even at minute concentrations, aflatoxins still pose serious health threats to humans. Liver necrosis could occur as a result of the consumption of high concentrations of aflatoxins and this is usually accompanied by rapid death (15).

Aflatoxins exist in four primary forms; B1, B2, G1, and G2. These forms occur in synergy, varying in proportions in foods. Since ambient humidity and plant moisture content are important factors in determining the growth and toxin output of these moulds, seed exposure occurs primarily in tropical and subtropical environments with high humidity and high temperatures. These atmospheric conditions are characteristic in parts of sub-Saharan Africa, the Asia Pacific region, and parts of South America. The probability for exposure is high in subsistence farming communities situated in these regions which experience these conditions, where regulations to control toxin exposure are either nonexistent or impracticable (16). Aflatoxin B_1 (AFB_1) has a similar geographical distribution to chronic HBV infection, colonizing various food products in the same Far Eastern and sub-Saharan African countries. Therefore, synergistic interactions of the hepatocarcinogenic effects of HBV and AFB_1 would possibly explain or elucidate the prevalence of HCC in these regions of the world.

Plate 2. Maize infested with *Aspergillus flavus* (17)

Probable modes of interaction between AFB_1 and HBV in hepatocarcinogenesis:

Some studies (as shown in Table 1) have been conducted to identify the possible mechanisms of synergism of AFB_1 and hepatitis B in the development of HCC. However, most of the studies were carried out decades ago, and there is currently inadequate information on the mechanisms of interaction between the primary causative factors. Some potential mechanisms have been suggested for the interaction of HBV and AFB_1 in the cause of HCC. A mechanism in which this is achieved is through specific cytochrome P450s that metabolize AFB_1 to AFB_1-8,9-epoxide, which may be caused either by chronic hepatitis by HBV infection or by the presence of the virus itself (Fig 3).

Induction of these phase I enzymes have been described in HBV transgenic mice (18). The hepatocyte damage caused by the virus seemed to result from this effect instead of the virus itself. The discovery that Gambian and Taiwanese children and adolescents chronically infected with HBV have lower AFB_1 adduct concentrations than non-infected people (19) correlate with this mechanism. The aflatoxin-8,9-epoxide generated has been shown to bind to proteins that cause acute toxicity or DNA changes that increase the probability of malignant transformation over time (20).

Table 1: Studies on hepatocarcinogenic synergy of aflatoxicosis and hepatitis B virus

Findings	Location	Reference
AFB_1 and HBV coinfection hastens the development of HCC	Swaziland, Africa	(21)
HCC prevalence is 10 times more in HBV-positive individuals with high aflatoxin consumption	Guangxi, China	(22)
HBsAg carriers with detectable aflatoxin-albumin adduct are likely to develop HCC	Taiwan, Asia	(23)
AFB_1 and HBV bind and integrate to *P53* protein, cause *P53* mutations and induce carcinogenesis	Ejura-sekyedumase District, Ghana	(24)
Exposure of AFB_1 can increase the risk of HCC through a dose-response among chronic HBV carriers.	Taiwan. Asia	(25)

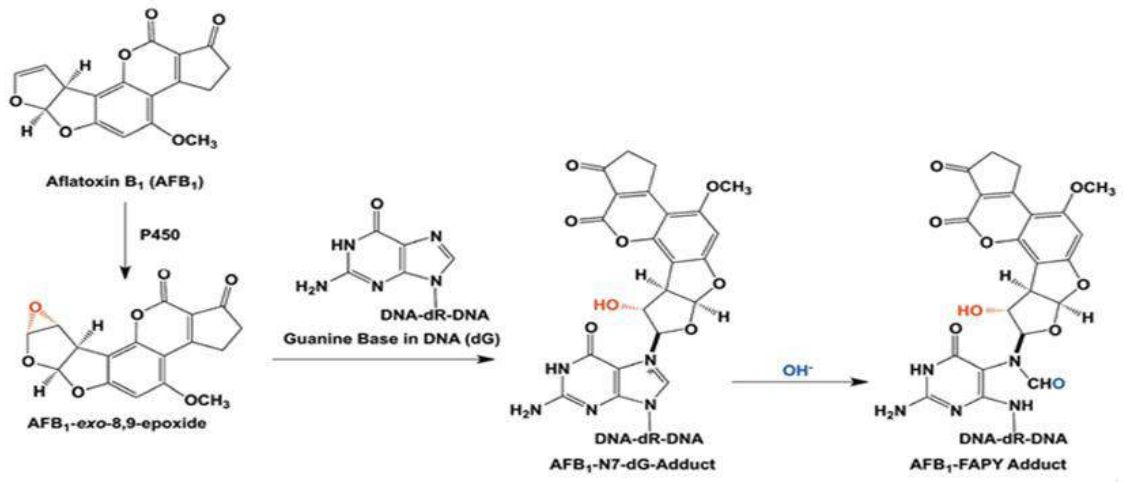


Fig 3: Diagrammatic illustration showing the metabolism of AFB_1 to AFB_1-8,9-epoxide to induce Guanine Base changes (26)

Another suggested hypothesis is that the development of phase II detoxification enzymes [glutathione S transferase (GST) and epoxy hydrolase (EPHX)] contribute to the proliferation of AFB_1 and HBV-induced HCC (23). There was a multiplicative association in the development of HCC in West African and Chinese patients between HBV infection and EPHX mutations (27); patients with chronic HBV infection but with healthy EPHX alleles were 15 times at higher risk, and those with both HBV infection and at least one EPHX mutant were at 77 times higher risk. Subsequent reports on these patients indicated a positive relationship between HBV and AFB_1 and which seemed to rely on the existence of a polymorphism of the genes GST T1, GST M1, and EPHX. These genes are normally responsible for transforming carcinogenic AFB_1-8,9-epoxide into non-reactive metabolites (23).

The carcinogenic association between AFB_1 and HBV may also be mediated through elevated hepatocyte necrosis, and proliferation due to chronic HBV infection, which increases the probability of both AFB1 mutations, like 249^{ser}, and eventual clonal expansion of cells carrying these mutations (28). Chronic necro-inflammatory hepatic conditions, like HBV infection contribute to generation of oxygen and nitrogen reactive species (29).

Prevention of HCC associated with AFB_1 and hepatitis B viral infection:

Genetics account for only 5–10% of all HCC cases, while environmental and lifestyle factors account for 90–95% of cancer cases (30). This suggests that the cancer can be prevented when risk factors that contribute to the development of cancer are avoided or reduced (31,32). A central concern of the public health sectors in developing countries is the HCC correlated with AFB_1. However, it remains a limiting factor in countries with weak public health and agricultural safety and food regulation systems. In countries with low capital, practical methods that have ensured minimal contamination of AFB_1 in industrialized countries cannot be applied realistically (33). Thus, efficient and effective approaches should be developed for developing countries to mitigate the AFB_1 contamination of human and animal foods. A variety of approaches have been tried (Fig 4) in order to reduce patient and population exposure to aflatoxins (34), also referred to as primary methods of prevention, as well as secondary forms of prevention using chemo-preventive agents to treat individuals at high risk of AFB_1 exposure (35).

It is necessary to implement long-term measures such as robust food safety systems to avoid hepatotoxicity and hepatocellular carcinogenesis induced by AFB_1. Steps should be at the level of individuals and communities. Such efforts must be directed at both market vendors and local farmers to avoid or reduce long-term exposure to aflatoxins, thereby increasing the incidence of HCC (36). Pre-harvesting measures include development of fungal-resistant crops and crops which have been genetically modified to inhibit the biosynthesis of aflatoxins and the application of insecticides and fungicides (35).

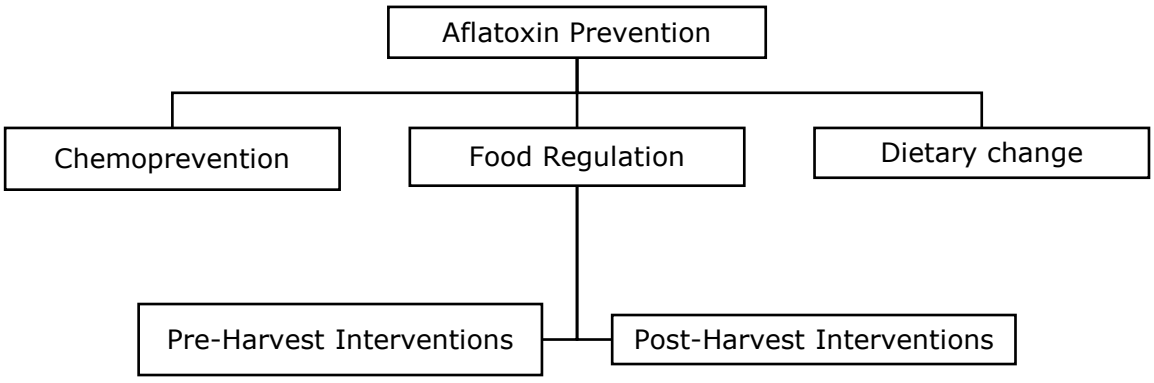


Fig 4: Proposed scheme for aflatoxin prevention (34)

To effectively prevent HBV infection, it is essential to understand its transmission route. Infection age and the origin of the disease are significant determinants in HBV infection outcome (37). The most important transmission route in endemic areas is perinatal mother-to-infant transmission; HBV infection occurs primarily during infancy and early childhood. In the absence of prophylaxis, 90% of babies develop chronic condition from highly infectious (HBeAg-positive) hepatitis B carrier mothers. HBV immunization can be categorized into passive and active immunization. Passive immunization with hepatitis B immunoglobulin (HBIG) provides temporary immunity, while effective vaccination provides long-term immunity. Since the primary infection route in endemic areas comes from the maternal transmission and the perinatal transmission results in a very high rate of chronic infection (90%), the best timing for initial HBV immunization should therefore be within 24 hrs of conception, accompanied by subsequent doses of HBV vaccine during infancy (38).

Other preventive measures, such as blood screening, proper sterilization of injection syringes, and avoidance of risky behaviors such as intravenous drug abuse, unprotected sex, skin piercing or tattooing can prevent horizontal transmission of HBV. Additionally, awareness should be provided to avoid high-risk behaviors such as encouraging condoms use during sexual intercourse. Most of the countries with low prevalence of HBV infection normally have adolescent HBV vaccinations to prevent exposure to sexual encounters or other threatening behaviors related to exposure to HBV.

Assessing further synergistic roles of AFB_1 and HBV in the development of HCC:

Although the etiological function of AFB_1 exposure to HCC has been researched for 50 years, there is limited information on the interaction of HBV infection (5). Hepatitis B virus is a significant risk factor in the development of primary liver cancer, and many of the studies are affected by this viral infection. In a study carried out by Lereau et al., (39), which sought to investigate the relationship between short-term exposure to AFB_1 (dose-dependent) and HBV on the *P53* gene in *HepaRG* cells, AFB_1 was evaluated as a natural antiviral agent. However, it was noted that there was no clear correlation between the doses applied to the cells in the study and the concentrations of AFB_1 liver after human dietary exposure.

AFB_1 research programs lack adequate resources in developing countries with high levels of aflatoxin food contamination in terms of qualified personnel, capital investment, and analytical and technical facilities. Besides, funding support for systematic assessments in these regions is essential in following all these factors. The truth is that much of society suffers from food shortages, so contaminated food products are likely consumed. For this reason, hepatitis B vaccine services have been proposed as a more practical and cost-effective plan to decrease the occurrence of liver cancer than to eliminate aflatoxin from the diet (40).

Incomplete and vague findings and hypotheses were made in regions where AFB1 and HBV are widely at risk for public health. For the prospective aspects of the research, a significant number of methodological optimizations and brand-new approaches are required, sustaining an apt opportunity to depict the profound effect of AFB_1. Researchers regarding this subject still face some issues. The primary problem is that most experiments have been redirected to endemic areas, showing high HBV rates, and the codon 249 mutation of the *p53* gene has not been tested to demonstrate any relation to the sensitivity to AFB_1. Another issue is that the accuracy of HCC clinical data and the prevalence of HBV are reduced. Also, the determination of AFB1 intake was not scrutinized for each patient and was restricted only to certain types of food (5).

From the findings of this mini-review, it is hoped that necessary action would be taken to monitor the aflatoxin levels and decrease the occurrence of aflatoxins in foods to benign levels. This is expected to increase the efforts by government and non-governmental organizations to support the course that would reduce the incidence of hepatitis B virus and aflatoxins-induced liver conditions.

Contribution of authors:

JOA, MOO, and OOB conceptualized the research; JOA, MOO, OOB, IOB, OJA, and OIK contributed to the development and writing of the manuscript; and JOA, MOO, and OOB contributed in validating and reviewing the manuscript. All authors approved submitted version.

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Original Article **Open Access**

Comparative evaluation of automated KingFisher Flex Purification System 96 (ThermoFisher Scientific) and manual QIAamp Viral RNA Mini Kit (Qiagen) extraction methods for SARS-CoV-2

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Abstract:

Background: The extraction step of the viral material of the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) influences the quality of reverse transcriptase-polymerase chain reaction (RT-PCR) results in diagnosis of coronavirus disease 2019 (COVID-19). The purpose of this cross-sectional study was to evaluate the diagnostic performance of the automated extraction system "KingFisher Flex Purification System 96 (ThermoFisher)" compared to the manual method with the "QIAamp Viral RNA Mini Kit (Qiagen)".
Methodology: From October to December 2020, comparative diagnostic evaluation of two methods of SARS-CoV-2 RNA extraction methods was conducted on 159 fresh and 120 frozen nasopharyngeal and oropharyngeal specimens collected from travellers and suspected cases or contacts of COVID-19 patients in Burkina Faso. The FastPlexTM Triplex 1-Step COVID 19 Detection Kit (RT-PCR, RNA extraction free) (Precigenome LLC) was used to amplify on the same PCR plate, RNA extracts from manual QIAamp Viral RNA Mini Kit and automated KingFisher Flex Purification System 96 (ThermoFisher) using the QuantStudio5 thermal cycler (Applied Biosystems). Analysis of the diagnostic performance of the SARS-CoV-2 RT-PCR assay following RNA extraction by the two methods was done using an online OpenEpi software.
Results: For fresh samples, the study found a slightly higher RT-PCR positivity rate following manual extraction (12.6%) than automated extraction (9.4%). For frozen samples, the positivity rate was far higher for manual (38.33%) than automated extraction method (20.83%). The results show that the performance of the automated extraction was inferior when compared to the manual extraction for both fresh samples (sensitivity 35%, specificity 94.2%) and frozen samples (sensitivity 43.5%, specificity 93.2%). However, using McNemar Chi-square with Yates correction, there was no significant difference in positivity rate of RT-PCR (x^2=0.76, p=0.38) between the two extraction methods for the fresh samples, but there was a significant difference (x^2=12.9, p= 0.0003) in the extraction of the frozen samples.
Conclusion: The results of this study showed that KingFisher Flex Purification System 96 (ThermoFisher) automatic extraction method was less sensitive and specific than QIAamp Viral RNA Mini Kit (Qiagen) manual extraction method. This information can serve as guide to laboratories in the choice of RNA extraction methods to use for RT-PCR detection of SARS-CoV-2.

Keywords: SARS-CoV-2; RNA extraction; Diagnostic; Performance; RT-PCR

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Évaluation comparative des méthodes d'extraction automatisée KingFisher Flex Purification System 96 (ThermoFisher Scientific) et manuelle QIAamp Viral RNA Mini Kit (Qiagen) pour le SRAS-CoV-2

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Résumé:

Contexte: L'étape d'extraction du matériel viral du syndrome respiratoire aigu sévère-coronavirus-2 (SRAS-CoV-2) influence la qualité des résultats de la réaction en chaîne de la transcriptase inverse-polymérase (RT-PCR) dans le diagnostic de la maladie à coronavirus 2019 (COVID-19). Le but de cette étude transversale était d'évaluer les performances diagnostiques du système d'extraction automatisé "KingFisher Flex Purification System 96 (Thermo- Fisher)" par rapport à la méthode manuelle avec le "QIAamp Viral RNA Mini Kit (Qiagen)"
Méthodologie: D'octobre à décembre 2020, une évaluation diagnostique comparative de deux méthodes d'extraction de l'ARN du SRAS-CoV-2 a été menée sur 159 échantillons nasopharyngés et oropharyngés frais et 120 échantillons congelés nasopharyngés et oropharyngés prélevés sur des voyageurs et des cas suspects ou des contacts de patients COVID-19 au Burkina Faso. Le kit de détection FastPlexTM Triplex COVID 19 (RT-PCR, sans extraction d'ARN) (Precigenome LLC) a été utilisé pour amplifier sur la même plaque PCR, des extraits d'ARN du kit manuel QIAamp Viral RNA Mini et du système automatisé KingFisher Flex Purification System 96 (ThermoFisher) à l'aide du thermocycleur QuantStudio5 (Applied Biosystems). L'analyse des performances diagnostiques du test SARS-CoV-2 RT-PCR après extraction de l'ARN par les deux méthodes a été effectuée à l'aide d'un logiciel OpenEpi en ligne.
Résultats: Pour les échantillons frais, l'étude a révélé un taux de positivité RT-PCR légèrement plus élevé après extraction manuelle (12,6%) qu'après extraction automatisée (9,4%). Pour les échantillons congelés, le taux de positivité était beaucoup plus élevé pour la méthode d'extraction manuelle (38,3%) que pour la méthode d'extraction automatisée (20,8%). Les résultats montrent que les performances de l'extraction automatisée étaient inférieures à celles de l'extraction manuelle pour les échantillons frais (sensibilité 35.0%, spécificité 94,2%) et les échantillons congelés (sensibilité 43,5%, spécificité 93,2%). Cependant, en utilisant McNemar Chi-carré avec correction de Yates, il n'y avait pas de différence significative dans le taux de positivité de la RT-PCR (x^2=0,76, p=0,38) entre les deux méthodes d'extraction pour les échantillons frais, mais il y avait une différence significative (x^2=12,9, p=0,0003) dans l'extraction des échantillons congelés.
Conclusion: Les résultats de cette étude ont montré que la méthode d'extraction automatique KingFisher Flex Purification System 96 (ThermoFisher) était moins sensible et spécifique que la méthode d'extraction manuelle QIAamp Viral RNA Mini Kit (Qiagen). Ces informations peuvent servir de guide aux laboratoires dans le choix des méthodes d'extraction d'ARN à utiliser pour la détection par RT-PCR du SRAS-CoV-2.

Mots-clés: SRAS-CoV-2; extraction d'ARN; diagnostique; performance; RT-PCR

Introduction:

Coronavirus disease 2019 (COVID-19) was declared a pandemic on March 11 2020 by the World Health Organization. The causative pathogen is the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (1,2). The molecular tests for detecting SARS-CoV-2 include reverse transcriptase-polymerase chain reaction (RT - PCR), transcription - mediated amplification (TMA), nicking enzyme-assisted reaction (NEAR), loop - mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and systems using clustered regularly interspaced short palindromic repeat (CRISPR-Cas) and next-generation sequencing (3). Also, antigen detection and serological tests are used for epidemiological study (3). RT-PCR is the 'gold standard' for COVID-19 diagnosis because it detects nucleic acid associated with genes such as spike (S), envelope (E), membrane (M), and nucleocapsid (N), open reading frame polyprotein (ORF1ab), and non-structural proteins such as NSP12 which encodes RNA-dependent RNA polymerase (RdRp) (4,5).

The quality of nucleic acid extraction and purification influences the sensitivity, reproducibility and accuracy of the RT-PCR test (6). In the last 10 years, several new manual, semi-automated and automated commercial nucleic acid extraction systems using magnetic beads or silica particles have been developed for DNA, RNA or total nucleic acid extraction (7). Thus, the magnetic separation extraction method uses a magnetic field to separate micrometer-sized paramagnetic particles from a suspension. The method is simple and reliable to purify several types of biomolecules, such as DNA, plasmids, RNA and proteins but requires more handling time (8,9). Furthermore, the extraction method with centrifugation columns uses purification materials such as glass fibre, silica and filter paper. The advantages of this method are ease of use, flexibility and automation capability (10,11). Manual nucleic acid extraction methods have limitations of contamination and inhibition. Contamination, in particular, is very possible in samples with high viral load in the early stages of SARS-CoV-2 infection (12,13)

Studies have compared some of these new extraction methods and reported that they differ in their ability to recover viral RNA, indicating that no single RNA extraction method is optimal for all viruses (14,15). Comparative studies of manual and automated nucleic acid extraction methods have been carried

on viruses such as rotavirus (16) and New Castle disease virus (17). A comparison study of six automated nucleic acid extraction systems (KingFisher ML, Biorobot EZ1, easyMAG, KingFisher Flex MagNA Pure Compact, Biorobot MDX) and one manual kit (Allprep DNA/ RNA Mini Kit) for respiratory pathogens reported that the systems differed in nucleic acid recovery, reproducibility, and linearity in a pathogen-specific manner (18).

In Burkina Faso, several extraction methods are used for SARS-CoV-2 RNA extraction but have not been formally compared to determine which is the most efficient. From the beginning of the pandemic in March 2020 in Burkina Faso, the "QIAamp Viral RNA Mini Kit (Qiagen)" was the first kit used by most laboratories involved in COVID-19 diagnosis. Then other manual kits such as MGIEasy Nucleic Acid Extraction Kit (MGI Tech Co., Ltd), MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems™), NUCLISENS® MINIMAG® (BioMérieux), Abbott Sample Preparation and automated kits such as MagNA PURE 96 and 24 (Roche), NucliSENS®easy MAG® 24 (BioMerieux), KingFisher Flex Purification System 24 and 96 (ThermoFisher), Arrow 12 (NorDiag/ DiaSorin), Abbott m2000 sp instrument 96 (Abbott) and abGenix™ 32 Nucleic acid extractor (AIT Biotech) (19). The QIAamp Viral RNA Mini Kit (Qiagen) produced more detectable RNA than the aforementioned kits (20).

After the acquisition of the automatic extractor (KingFisher) im our laboratory, we wanted to compare its performance to those of "QIAamp Viral RNA Mini Kit (Qiagen)", which prompted its choice as a reference. The main objective of this study therefore was to evaluate the performance of the automated extraction system "KingFisher Flex Purification System 96 (ThermoFisher)" in comparison to the manual method with the "QIAamp Viral RNA Mini Kit (Qiagen) in the perspective of its routine use.

Materials and method:

Study setting and design:

The study is a comparative evaluation of two RNA extraction methods for the *in vitro* detection of SARS-CoV-2, conducted between April and August 2021 in the Biomedical Research Laboratory (LaReBio) at the Institute for Research in Health Sciences (IRSS/CNRST), Ouagadougou. This is one of the laboratories involved in the COVID 19 diagnosis in Burkina Faso.

Ethics approval:

The Ministry of Health/Burkina Faso approved the evaluation of COVID-19 tests with the letter number N°2020/00004382/MS/ SG/DGAP/DLBM/sc dated 28 December 2020. It was carried at LaReBio, as recommended by the quality management system to any new method. This technical validation study is a contribution to the improvement of COVID-19 diagnosis in the laboratory. All the samples used under anonymous and confidential.

Nature and origin of the samples:

Nasopharyngeal and/or oropharyngeal specimens, including 159 fresh specimens [collected into viral transport medium (VTM) less than 24 hours and stored at 4-8°C] and 120 frozen specimens (collected into VTM from October to December 2020, stored at -80°C). All the samples were from the travellers' sites (airport, CMA Kossodo and IRSS) and suspected cases or contacts of COVID-19 patients.

Sample analysis methods:

All samples were extracted in duplicate using the two extraction methods; manual "QIAamp Viral RNA Mini Kit (Qiagen, Germany) and automated "KingFisher Flex" (ThermoFisher Scientific, USA). The amplification of both RNA extracts was done on the same PCR plate as shown in the flow chart (Fig 1).

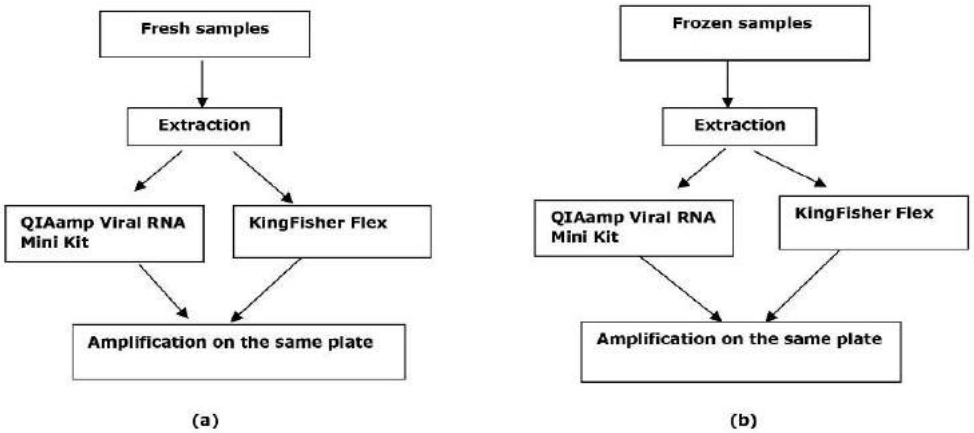

Fig 1: Flowchart of the analysis steps for fresh (a) and frozen (b) samples

The manual method "QIAamp Viral RNA Mini Kit" uses centrifugation columns on which a silica matrix is fixed. The principle is to lyse the cells to isolate the nucleic acid, attach it to a silica matrix, remove all contaminants by washing and elute the purified nucleic acid in a buffer. The KingFisher Flex Purification System 96 (ThermoFisher) is an automated extraction instrument. It provides consistent high-throughput extraction and purification of DNA, RNA, proteins and cells. It uses the MagMAX™ Viral/Pathogen Kit (ThermoFisher Scientific, USA) based on magnetic bead technology, designed to isolate and purify viral RNA and DNA.

RNA RT-PCR assay:

The FastPlexTM Triplex 1-Step COVID-19 Detection Kit (RT-PCR, RNA extraction free) (Precigenome LLC) was used to amplify on the same PCR plate the RNA extracts from the QIAamp Viral RNA Mini Kit and the KingFisher Flex Purification System 96 (ThermoFisher) using the QuantStudio5 thermal cycler (Applied Biosystems). The targets by the amplification kits are; ORF1ab (FAM fluorochrome) and N (HEX fluorochrome) and the internal control (CY5 fluorochrome) (Table 1).

Table 1: Interpretation of RT-PCR results

ORF1ab (FAM)	N (HEX)	IC (CY5)	Results
+	+	Not considered	SARS-CoV-2 positive
+	-		
-	+	+	SARS-CoV-2 Negative
-	-	-	Invalid

Negative result: Ct value > 39; Positive result: Ct value ≤ 39; Invalid: Ct >39 or no Ct detected for internal control (all samples' internal control)

Statistical analysis:

Data were entered into Excel 2016 and analyzed on R software. The mean Ct values of SARS-CoV-2 RT-PCR following manual and automated extraction methods were compared using the student's '*t*' test and the significance level was set at $p<0.05$. The sensitivity, specificity, positive predictive and negative predictive values of RT-PCR following automated KingFisher Flex extraction was calculated using the QIAamp manual method as the 'gold standard' with Open Epi. (http://www.openepi.com/Menu/OE_Menu.htm).

Results:

Characteristics of patients with fresh samples:

The mean age of the patients was 40.26 ± 12.89 years (age range 5 - 74 years). The age group 30-40 years was in the majority (32.07%), followed by age group 40-50 years with 25.79%. Most patients (66.67%) were male with gender ratio of 2.95. The most frequent reason for sample collection was travel at 50.94%, followed by contact cases of SARS-CoV-2 infected patients at 19.49%. The majority (95.60%) of the patients resided in Ouagadougou and the rest were undefined.

Characteristics of patients with frozen samples

The mean age of patients was 34.87± 14.51 years (age range 11-78 years). The age group 20-30 years was the most represented (26.67%), followed by age group 30-40 years with 22.5%. Most of the patients were male (60%). The most frequent reason for testing was "contact" of SARS-CoV-2 cases (49.16%), followed by travelers (45%) and others (suspected cases and controls). Almost all (96.7%) of the patients resided in Ouagadougou and the samples were collected from the Ouagadougou sampling sites.

Evaluation of the automated and manual extraction methods for fresh samples:

The study found a slightly higher RT-PCR positivity rate of fresh samples following manual extraction (12.6%, 20/159) than automated extraction (9.4%, 15/159). Seven (4.4%) samples were positive following both manual and automated extraction while 131 (82.4%) were negative. Compared to manual extraction, automated extraction had a specificity of 94.2%, sensitivity of 35.0%, PPV of 46.7% and NPV of 90.9% for fresh samples but using McNemar Chi-square with Yates correction, there was no significant difference between both methods of extraction ($x^2=0.76$, $p=0.38$) with the fresh samples (Table 2a).

Evaluation of the automatic and manual extraction method for frozen samples:

For the frozen samples, there was a significant difference in the RT-PCR positivity rate following manual extraction (38.33%, 46/120) and automated extraction (20.83%, 25/120) ($x^2=12.9$, $p=0.0003$). Twenty (16.7%) samples were positive following both manual and automated extraction and 69 (57.5%) samples were negative following both extraction methods. Compared to manual extraction, automated extraction had a specificity of 93.24%, sensitivity of 43.48%, PPV of 80.0% and NPV of 72.63% (Table 2b).

Table 2a: Comparative evaluation of RT-PCR results following automatic and manual extraction methods from fresh samples

RNA extraction results/samples		**Nasopharyngeal samples**		**Oropharyngeal samples**		**Total**
		Positive	**Negative**	**Positive**	**Negative**	
Results of the automatic extraction	Positive	7	7	0	1	15
	Negative	13	128	0	3	144
	Total	20	135	0	4	159
	Sensitivity (95% CI)	35.00% (18.12 - 56.71)				
	Specificity (95% CI)	94.24% (89.05 - 97.06)				
	PPV (95% CI)	46.67% (24.81 - 69.88)				
	NPV (95% CI)	90.97% (85.17 - 94.65)				
	Kappa de Cohen coefficient (95% CI)	0.33 (0.17 - 0.48)				
	McNemar with Yeates correction	x^2= 0.76, p=0.38				

PPV: Positive predictive value, NPV: negative predictive value, 95% CI: 95% confidence interval

Table 2b: Comparative evaluation of RT-PCR results following automatic and manual extraction methods from frozen samples

RNA extraction results/samples		**Nasopharyngeal samples**		**Oropharyngeal samples**		**Total**
		Positive	**Negative**	**Positive**	**Negative**	
Results of the automatic extraction	Positive	13	4	7	1	25
	Negative	19	54	7	15	95
	Total	32	58	14	16	120
	Sensitivity (95%CI)	43.48% (30.21 - 57.75)				
	Specificity(95%CI)	93.24% (85.14 - 97.08)				
	PPV (95% CI)	80.0% (60.87 - 91.14)				
	NPV (95% CI)	72.63% (62.92 - 80.59)				
	Kappa de Cohen Coefficient (95%CI)	0.40 (0.23 - 0.56)				
	McNemar with Yeates correction	x^2= 12.90, p=0.0003				

PPV: Positive predictive value, NPV: negative predictive value, 95% CI: 95% confidence interval

Table 3: Mean cycle threshold (C_t) values in RT-PCR of SARS-COV-2 genes following RNA extractions from fresh and frozen samples

Fresh samples				
Targets	**QIAamp C_t (mean± SD)**	**KingFisher Flex C_t (mean± SD)**	**C_t difference (C_t Kingfisher - C_t QIAamp)**	**p value**
ORF1ab	31.32 ± 3.41	33.68 ± 2.76	+2.36	<0.0001*
N	33.74 ± 4.69	34.55 ± 4.87	+ 0.81	0.1319
IC	26.88 ± 3.69	26.6 ± 3.60	-0.28	0.4939
Frozen samples				
Targets	**QIAamp C_t (mean± SD)**	**KingFisher Flex C_t (mean± SD)**	**C_t difference (C_t Kingfisher - C_t QIAamp)**	**p value**
ORF1ab	30.74± 5.98	30.73± 4.65	-0.01	0.9885
N	33.04± 4.36	32.04± 3.80	-1	0.0594
IC	27.18± 4.00	27.96± 3.39	+0.78	0.1045

ORF1ab: Open reading frame1ab, N: nucleocapsid protein, IC: internal control; *: statistically significant difference

Comparison of mean C_t values of different SARS-CoV-2 genes following manual and automated extraction of fresh samples:

There was a gain of 2.36 and 0.81 cycles of amplification for the ORF1ab and N gene respectively for KingFisher Flex automated over the QIAamp extraction method from the fresh samples with a statistically significant difference between the mean C_t values for the ORF1ab gene (p<0.0001), but there was no significant difference between the mean C_t values for the N gene (p=0.1319) between the manual and automated extraction methods. Also, there was no significant difference between the mean C_t values of the internal control between the manual and automated extraction (p=0.4939), although there was a loss of threshold cycle (C_t) of -0.28 cycles for the internal control (Cy5) with the KingFisher Flex automated method compared to QIAamp manual extraction method (Table 3).

Comparison of mean C_t values of different SARS-CoV-2 genes following manual and automated extraction of frozen samples:

There was no statistically significant difference in the mean C_t value of the ORF1ab gene between the manual and automated extraction methods (p=0.9885) although there was a loss of -0.01 amplification cycle by the automated method. Similarly, there was no significant difference between the mean C_t values of the N gene between the manual and automated extraction (p=0.0594) although there was a loss of one amplification cycle (-1 cycle) by the automated extraction method. Also, there was no significant difference between the mean C_t of the internal control following manual and automated extraction (p= 0.1045) although there was a gain of +0.78 cycles by the automated KingFisher Flex over manual QIAamp extraction method (Table 3).

Discussion:

This study compared an automated extraction method for SARS-CoV-2 RNA with a manual method, used as reference. It shows that compared to the "QIAamp Viral RNA Mini Kit (Qiagen)", the "KingFisher Flex Purification System 96 (ThermoFisher)" automated extraction method loses some performance. Indeed, the study found a slightly higher SARS-CoV-2 RT-PCR positivity rate for fresh samples for manual extraction (12.6%) than for automatic extraction (9.4%). For frozen samples, there was also difference in the positivity rate following manual extraction (38.3%) than automatic extraction (20.8%). A previous study (21) showed that there was no statistically significant difference (p=0.629) in the RT-PCR positivity rate (92.5% vs 90%) between manual and automated methods. This may be explained by the fact that the methods used are different. In this previous study, automated liquid-based high-throughput RNA extraction platform (PHASIFY™) was compared with the widely used magnetic bead-based total nucleic acid extraction (MBTE) platform (NucliSENS ® easyMAG ®).

Our study shows that automatic extraction on fresh and frozen samples was more specific (94.24%, 93.24%) but less sensitive (35%, 43.48%) in detecting SARS-CoV-2 on RT-PCR. Using the 2x2 contingency table analysis, manual extraction was more sensitive than automated extraction, and although this

was not statistically significant for fresh samples on McNemar Chi-square with Yates correction (x^2=0.76, p=0.38), it was statistically significant for frozen samples (x^2=12.9, p= 0.0003). Contrariwise, researchers in Brazil (22) found that automated extraction (Loccus, Extracta Kit FAST) was the most sensitive (100%) compared to manual extraction (Bio-Gene Kit, Bioclin, Quibasa) and rapid extraction methods (Lucigen, Quick DNA Extract Kit). This discordant finding could be explained by the different extraction techniques used in these studies.

The mean cycle threshold (C_t) value of the RT-PCR for SARS-CoV-2 ORF1ab gene for fresh samples was significantly higher with the KingFisher Flex extraction (p<0.0001) than manual QIAamp Viral RNA Mini Kit extracts, indicating that manual extraction was more sensitive in detecting the ORF1ab gene from fresh samples, but there was no significant difference in the C_t for the N gene (p=0.1319) between the two methods. For the frozen samples, the mean C_t values of SARS-CoV-2 ORF1 ab (p=0.9885) and N genes (p=0.0594) were not significantly different for both manual QIAamp Viral RNA Kit and automated KingFisher Flex extraction methods.

The results obtained for the fresh samples in our study agrees with those of Esona et al., (16) who in comparing two automated methods found that the mean C_t values for the KingFisher Flex extracts were significantly higher (p=0.001) than those of the other methods (MagNA Pure Compact or RNaid kit). However, our results of the frozen samples contrast those of Ransom et al., (23) who in their study found that the mean C_t values of RT-PCR with the KingFisher Flex were significantly lower (p=0.05) than those of the EZ1 and easyMAG, and although there was a loss of 0.01 and 1 cycle for ORF1ab and N genes respectively with the KingFisher Flex extraction method for the frozen samples, which may indicate higher sensitivity of SARS-CoV-2 detection, these losses did not reach statistical significance (p>0.05). Nevertheless, our results showed that manual QIAamp Viral RNA Mini Kit, compared to KingFisher Flex Purification System 96, was more sensitive for the extraction of ORF1ab RNA from fresh samples than from frozen samples.

Furthermore, it has been extrapolated in a study (24) that each 3.3 increase in C_t value corresponds to approximately 1 log (i. e. 10-fold) less target in the primary clinical specimen subjected to PCR reaction. Similarly, some researchers have attempted to correlate C_t values with SARS-CoV-2 detection (25), thus gains (e. g. gain of 2.36 cycles from the manual over automatic for the ORF1ab gene in the fresh samples in our study) or losses (e. g. loss of -0.01 cycles from manual to automatic for the ORF1ab gene in the frozen samples) of C_t can influence "positivity" or "negativity" of RT-PCR test. It appears that automated extraction may be better suited for frozen than fresh samples, as the target genes are detected earlier. But our findings remain preliminary and requires to be validated in a more comprehensive study comparing the two methods.

Our study is limited by the non-repeatability of the extraction and RT-PCR assays on the tested samples, lack of information and data on the symptoms of the tested subjects and possible contamination with manual extractions. Nonetheless, the results of our study showed that automated RNA extraction with KingFisher Flex was less sensitive for RT-PCR detection of SARS-CoV-2 from fresh samples than manual extraction with QIAamp kit. This finding could serve as guide for laboratories in selecting extraction methods based on periods of COVID-19 sampling and target individuals to be diagnosed.

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Contributions of authors:

AAZ conceptualized the study and was involved in data curation, formal analysis, software, and writing original manuscript draft; HGO was involved in study conceptualization, laboratory analysis, supervision, review and manuscript editing; TRC was involved in data curation, review and manuscript editing; JAB was involved in data curation, review and manuscript editing; TS was involved in laboratory analysis, validation, review and manuscript ed iting; STS was involved in laboratory analysis, review and manuscript editing; CD was involved in data curation; ARN was involved in data curation; and NB was involved in supervision and data validation.

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Conflicts of interest:

No conflict of interest is declared.

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Original Article **Open Access**

Prevalence of COVID-19 at the Wahgnion-Gold mining site in Burkina Faso and use of RT-PCR initial cycle threshold to monitor the dynamics of SARS-CoV-2 load

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Abstract:

Background: To control the spread of coronavirus disease-19 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), it is necessary to adequately identify and isolate infectious patients particularly at the work place. Real time polymerase chain reaction (RT-PCR) assay is the recommended confirmatory method for the diagnosis of SARS-CoV-2 infection. The aim of this study was to determine the prevalence of SARS-CoV-2 infection in Burkina Faso and to use the initial cycle threshold (Ct) values of RT-PCR as a tool to monitor the dynamics of the viral load.
Methodology: Between September 2021 and February 2022, oropharyngeal and/or nasopharyngeal swab samples of consecutively selected COVID-19 symptomatic and apparently healthy workers from the Wahgnion mining site in the South-western Burkina Faso who consented to the study were collected according to the two weeks shift program and tested for SARS-CoV-2 using RT-PCR assay. Patients positive for the virus were followed-up weekly until tests were negative. Association of the initial RT-PCR Ct values with disease duration was assessed by adjusted linear regression approach. Two-sided *p* value < 0.05 was considered statistically significant.
Results: A total of 1506 (92.9% males) participants were recruited into the study, with mean age and age range of 37.1±8.7 and 18-68 years respectively. The overall prevalence of SARS-CoV-2 infection was 14.3% (216/1506). Of the 82 patients included in the follow-up study, the longest duration of positive RT-PCR test, from the first positive to the first of the two negative RT-PCR tests, was 33 days (mean 11.6 days, median 10 days, interquartile range 8-14 days). The initial Ct values significantly correlated with the duration of RT-PCR positivity (with β=-0.54, standard error=0.09 for N gene, and β=-0.44, standard error=0.09 for ORF1ab gene, $p<0.001$). Participants with higher Ct values corresponding to lower viral loads had shorter viral clearance time than those of lower Ct values or higher viral loads.
Conclusion: Approximately 1 out of 7 tested miners had SARS-CoV-2 infection and the duration of their RT-PCR tests positivity independently correlated with the initial viral load measured by initial Ct values. As participants with lower initial Ct values tended to have longer disease duration, initial RT-PCR Ct values could be used to guide COVID-19 patient quarantine duration particularly at the work place.

Keywords: SARS-CoV-2, COVID-19, RT-PCR, cycle threshold, prevalence, Burkina Faso

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Prévalence du COVID-19 sur le site minier de Wahgnion-Gold au Burkina Faso et utilisation du seuil de cycle initial de RT-PCR pour surveiller la dynamique de charge du SARS-CoV-2

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Résumé:

Contexte: Pour contrôler la propagation de la maladie à coronavirus 19 (COVID-19) causée par le syndrome respiratoire aigu sévère coronavirus-2 (SRAS-CoV-2), il est nécessaire d'identifier et d'isoler de manière adéquate les patients infectieux, en particulier sur le lieu de travail. Le test de réaction en chaîne par polymérase en temps réel (RT-PCR) est la méthode de confirmation recommandée pour le diagnostic de l'infection par le SRAS-CoV-2. Le but de cette étude était de déterminer la prévalence de l'infection par le SRAS-CoV-2 au Burkina Faso et d'utiliser les valeurs du seuil initial du cycle (Ct) de la RT-PCR comme outil de suivi de la dynamique de la charge virale.
Méthodologie: Entre septembre 2021 et février 2022, des écouvillonnages oropharyngés et/ou nasopharyngés de travailleurs symptomatiques COVID-19 et apparemment en bonne santé sélectionnés consécutivement du site minier de Wahgnion dans le sud-ouest du Burkina Faso qui ont consenti à l'étude ont été prélevés selon les deux programme de quart de semaines et testé pour le SRAS-CoV-2 à l'aide d'un test RT-PCR. Les patients positifs pour le virus ont été suivis chaque semaine jusqu'à ce que les tests soient négatifs. L'association des valeurs Ct initiales de la RT-PCR avec la durée de la maladie a été évaluée par une approche de régression linéaire ajustée. Une valeur p bilatérale < 0,05 a été considérée comme statistiquement significative.
Résultats: Un total de 1506 participants (92,9% d'hommes) ont été recrutés dans l'étude, avec un âge moyen et une tranche d'âge de 37,1 à 8,7 ans et de 18 à 68 ans, respectivement. La prévalence globale de l'infection par le SRAS-CoV-2 était de 14,3% (216/1506). Sur les 82 patients inclus dans l'étude de suivi, la plus longue durée de test RT-PCR positif, du premier test positif au premier des deux tests RT-PCR négatifs, était de 33 jours (moyenne 11,6 jours, médiane 10 jours, intervalle interquartile 8-14 jours). Les valeurs Ct initiales étaient significativement corrélées à la durée de positivité de la RT-PCR (avec β=-0,54, erreur standard=0,09 pour le gène N et β=-0,44, erreur standard=0,09 pour le gène ORF1ab, p<0,001). Les participants avec des valeurs de Ct plus élevées correspondant à des charges virales plus faibles avaient un temps de clairance virale plus court que ceux avec des valeurs de Ct plus basses ou des charges virales plus élevées.
Conclusion: Environ 1 mineur testé sur 7 était infecté par le SRAS-CoV-2 et la durée de la positivité de ses tests RT-PCR était indépendamment corrélée à la charge virale initiale mesurée par les valeurs Ct initiales. Comme les participants avec des valeurs Ct initiales inférieures avaient tendance à avoir une durée de maladie plus longue, les valeurs Ct initiales de la RT-PCR pourraient être utilisées pour guider la durée de la quarantaine des patients COVID-19, en particulier sur le lieu de travail.

Mots clés: SRAS-CoV-2, COVID-19, RT-PCR, seuil de cycle, prévalence, Burkina Faso

Introduction:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing the 2019 coronavirus disease pandemic (COVID-19) was first identified in December 2019 in Wuhan, China (1,2). The outbreak subsequently spread worldwide, affecting over 522 million persons globally with approximately 6.25 million deaths as of May 22, 2022 (3,4). The first case in Burkina Faso was identified on March 9, 2020, and as of 20 June 2022, 21,044 cases and 387 deaths were reported according to the country's health authority records (5).

To counter the spread of the disease and limit its public health implications, the World Health Organization (WHO) cautioned the public to take responsive care and the public care strategies have included handwashing, wearing of face masks, physical distancing, avoiding mass gathering and assemblies, and total lockdowns, which have flattened the transmission curve allowing the reopening of public services (3). Testing has been crucial to track the spread of the disease during the pandemic, and to swiftly implement the needed public health interventions such as isolation, quarantine, and appropriate clinical management of cases.

Real-time reverse-transcription polymerase chain reaction (RT-PCR) test, which is a nucleic acid amplification test (NAAT) to detect viral nucleic acid, has routinely been used to detect SARS-CoV-2 in oropharyngeal or nasopharyngeal swabs of individuals suspected of COVID-19, and currently represents the 'gold standard' diagnostic methods for COVID-19 (6). Different viral target gene structures, including the nucleocapsid (N) and the open reading frame (ORF) 1 (O) gene, have been used for the diagnosis of SARS-CoV-2 infection (7). During

the amplification process in RT-PCR assay, cycle threshold (Ct) values are used to assess the viral nucleic acid amplification for the target gene structure, and values that cross the threshold are used to discriminate positive from negative samples (8).

Although case diagnosis and optimal quarantine strategies have been an important component for the public health strategies of SARS-CoV-2 transmission prevention (9), the procedure consists of several steps, and needs laboratory equipment that makes the process tedious and difficult to be conducted outside the laboratory setting particularly in resource constraint settings (10). Thus, finding ways to reduce the burden of samples to be tested would substantially reduce the work load, and thus allow a more efficient use of resources in sub-Saharan African countries.

Exploring the relationship between the initial PCR Ct values could tentatively indicate the duration of the PCR test positivity, and thus help eliminate unnecessary testing. However, research studies assessing the association of the initial PCR Ct values and the duration of the PCR test positivity are uncommon and included very limited sample size which make the estimates less precise (11). The aim of this study is to determine the prevalence of SARS-CoV-2 infection at a mining site in Burkina Faso, and assess the initial PCR Ct values as an indirect measure of the dynamics of the viral load in COVID-19 patients.

Materials and method:

Study area and design

This was a prospective surveillance study integrated to the work shift of mine workers at the Wahgnion-Gold mining site in south-western Burkina Faso. Miners were required a negative PCR test before returning to their work place. All apparently healthy or COVID-19 symptomatic miners who consented to participate and adhere to the study procedures were systematically included in a consecutive and sequential manner as they visited the COVID-19 testing center located on the mining site over the study period between August, 2021 and January, 2022.

Ethics approval and consent to participate

This was a pandemic response/surveillance data and the study protocol ethical clearance was obtained from the national ethics committee of health research of Burkina Faso (clearance certificate number CERS-2020-7-126). In addition, data were fully anonymized to protect participants' identities and personal data. Usage was done in accordance ethical regulations.

Sample and data collection

For each participant, oropharyngeal (OP) or nasopharyngeal (NP) swabs were collected, and participants socio-demographic and medical characteristics were collected using interviewer-administered data collection form. All patients tested positive for SARS-CoV-2 were included in the follow-up study if they agreed to participate and had their follow-up swabs assessed by RT-PCR. Follow up swabs were collected each 3-7 days interval according to the patient availability until two consecutive negative RT-PCR test results were obtained.

Sample management and RNA extraction

Samples were stored in universal transport medium (Copan Diagnostics) and transferred to the National Influenza Reference Laboratory (NIRL) for analysis. Viral RNA was manually extracted from 200µL of virus transport medium containing NP and OP swabs by using QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany), and eluted into 60 µL elution buffer according to the manufacturer's instruction. The eluted RNAs (templates for RT-PCR) were stored immediately at minus 80°C until use.

SARS-CoV-2 RT-PCR assays

Two different real-time reverse transcription polymerase chain reaction (rRT-PCR) assays; the Viasure SARS-CoV-2 RT-PCR detection kit (Certest Biotec SL., Spain) and the detection kit for 2019 novel coronavirus (2019-nCoV) RNA (PCR-fluorescence probing) manufactured by Da An Gene Co., Ltd. of Sun Yat-sen University, were used to detect N and ORF 1ab genes using the Applied Biosystems 7500 RT-PCR instrument (Thermo Fisher Scientific). The Da An Gene co kit was used for initial detection of COVID-19 positive subjects. Positive samples were further retested with the Viasure kit to establish the baseline cycle threshold (Ct) values and patients were followed up until the time of the first negative test.

The detection kit for 2019 novel coronavirus (2019-nCoV) RNA of Da An Gene Co., Ltd has the 2019-nCoV primer and probe sets designed to detect N and ORF1ab gene sequences. Further, human housekeeping gene RNP (Ribonuclease P) was developed as the target gene for the internal control for monitoring the specimen collection, nucleic acid extraction and PCR amplification processes, to reduce occurrence of false negative results. Each 25µL of reaction mix contained 17µL of NC (ORF1ab/N) PCR reaction solution A, 3µL of NC (ORF1ab/N) PCR reaction solution B, and 5µL of RNA. Thermal cycling was performed at 50°C for 15min and 95°C for 15

min followed by 45 cycles of 94°C for 15 seconds and 55°C for 45 seconds. Each run included NC (ORF1ab/N) negative control and NC (ORF1ab/N) positive control. Specimens with a cycle threshold (Ct) value ≤ 40 for SARS-CoV-2 ORF1ab and N gene targets were considered positive.

The Viasure SARS-CoV-2 RT-PCR detection kit contains in each well of the PCR plate all the components necessary for RT-PCR assay (specific primers/probes, dNTPs, buffer, polymerase and retro-transcriptase) in a stabilized format, as well as an internal control to monitor PCR inhibition. Briefly, each 20µL of reaction mix contained 15µL mix of enzymes, primers, probes, buffer, dNTPs, stabilizers and internal control in stabilized format which is reconstituted of the rehydration Buffer and 5µL of RNA. Thermal cycling was performed at 45°C for 15min and 95°C for 02 m, followed by 45 cycles of 95°C for 10 seconds and 60°C for 50 seconds. Each run included reconstituted SARS-CoV-2 positive control and negative control.

A sample is considered positive if the Ct value obtained is less than 38 for SARS-CoV-2 ORF1ab and N genes or ORF1ab SARS-CoV-2 gene only and the internal control shows or not an amplification signal. If N target only was positive, the interpretation was SARS-CoV-2 presumptive positive and additional confirmatory testing was conducted by the reference laboratory.

Statistical analysis

Data were entered onto an Excel database and transferred onto R statistical software (R Development Core Team, 2021) for statistical analysis. We carried out a patient-level analysis by first describing the distribution of their sociodemographic and medical characteristics. To assess the association of patients COVID-19 initial RT-PCR Ct values with duration of the illness, we calculated the duration as the difference between the initial diagnosis date and the date of the first of two negative test results. Next, we modeled the association using adjusted general linear regression models to calculate effect estimates and 95% confidence interval (CI) per Ct value. Means were compared by the *t*-test and medians were compared by Wilcoxon exact test. Statistical significance was determined at two-sided p value < 0.05.

Results:

Characteristics of study participants

A total of 1506 participants were tested, with a male predominance (ratio of 13:1), mean age of 37.1 ± 8.7 years and age range of 18-68 years. The city of Bobo-Dioulasso was the most represented residency area (58.2%) with the others, representing 19.5%, 10.6%, 7.5%, and 2.5% for Niankorodougou, Sindou, Banfora, and Ouagadougou respectively. Majority of the study participants (82.7%) were nationals from Burkina Faso with others (17.3%) being expatriate workers.

The oropharyngeal and nasopharyngeal swabs represented 60.2% and 39.6% respectively of the total samples and two participants had mix (nasopharyngeal and oropharyngeal) swabs (Table 1).

Prevalence of SARS-CoV-2 infections

The overall prevalence of SARS-CoV-2 infection was 14.3% (216/1506). Participants from the cities of Sindou, Bobo-Dioulasso, and Niankorodougou, were the most infected with 22.6%, 15.3% and 13.3% of their respective total participants. The prevalence of SARS-CoV-2 infections was not significantly different according to participants gender (13.1% for female and 14.4% for male, p=0.9) or the type of swab samples collected (18.4% vs 11.7%, p=0.32).

Cycle threshold values and duration of disease

Of the 82 participants positive for SARS-CoV-2 and who had RT-PCR follow-up data available, the median duration from initial positive PCR test results to the first results of the two consecutive negative PCR results was 10 days, with range of 3-33 days. Half of the participants had their duration between 8 and 14 days (first and third quartiles). The association of initial Ct values with the duration of the illness is shown in Fig 1 for the N-gene and Fig 2 for the ORF 1ab gene.

The duration of positive RT-PCR test was negatively correlated with the initial Ct value. The longest duration of positive RT-PCR was 33 days from the first positive RT-PCR test results. The mean duration was 11.6 days from initial positive RT-PCR test to the first of the two consecutive negative RT-PCR tests, and for each additional Ct value, the average illness duration significantly decreased by 0.54 day for the N-gene and 0.44 day for the ORF1ab gene (p< 0.001) when adjusted with the patient age, gender, and type of samples (nasopharyngeal or oropharyngeal).

Table 1: Characteristics of study participants at the Wahgnion-Gold mining site in Burkina Faso

Characteristics	Measured statistic
Age (years)	
Mean age (mean ± SD)	37.1± 8.7
Age range	18-68
Gender	
Male, n (%)	1399 (92.9)
Female, n (%)	107 (7.1)
Origin country	
National n, (%)	1245 (82.7)
Expatriate n, (%)	261 (17.3)
Residency	
Bobo Dioulasso, n (%)	877 (58.2)
Sindou, n (%)	159 (10.6)
Banfora, n (%)	113 (7.5)
Niankorodougou, n (%)	293 (19.5)
Ouagadougou, n (%)	37 (2.5)
Others, n (%)	27 (1.8)
Type of swabs	
Oropharyngeal, n (%)	907 (60.2)
Nasopharyngeal, n (%)	597 (39.6)
Mix (oropharyngeal and nasopharyngeal), n (%)	2 (0.1)
Cycle threshold at first PCR test	
N-gene, (median, range)	26.57 (11.68 -38.24)
ORF1ab gene, (median, range)	25.43 (13.79-40)

SD = standard deviation; PCR = polymerase chain reaction; Ct = cycle threshold; n= total count, % = percentage

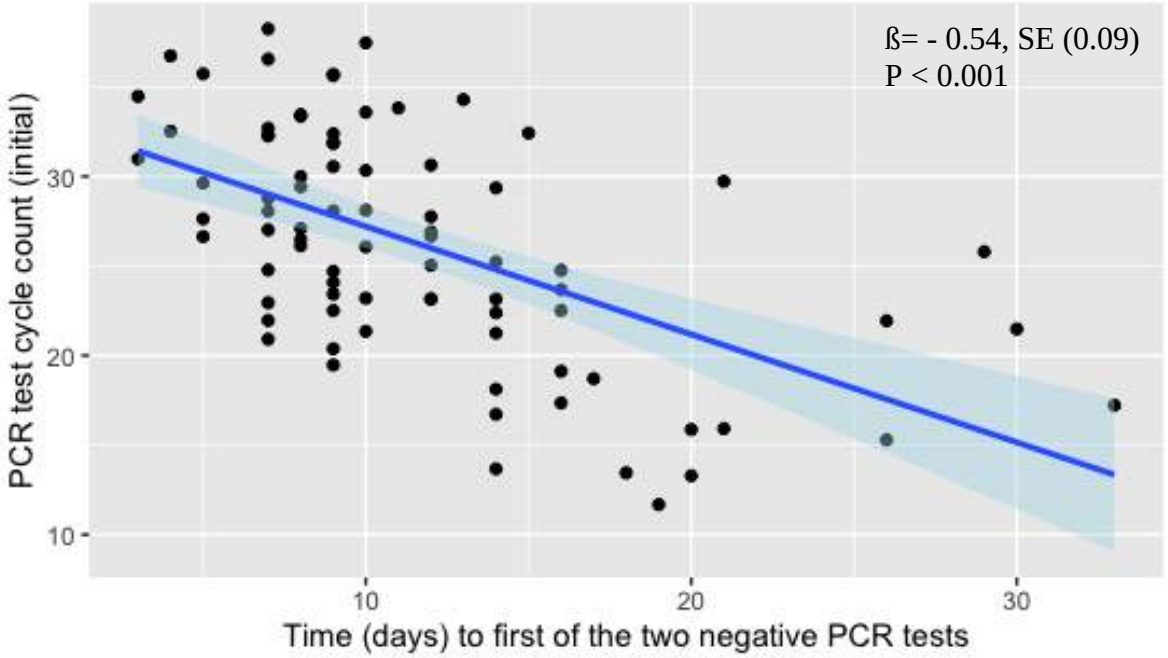

Fig 1: Duration of positive SARS-CoV-2 RT-PCR test according to initial cycle threshold (Ct) value for N-gene

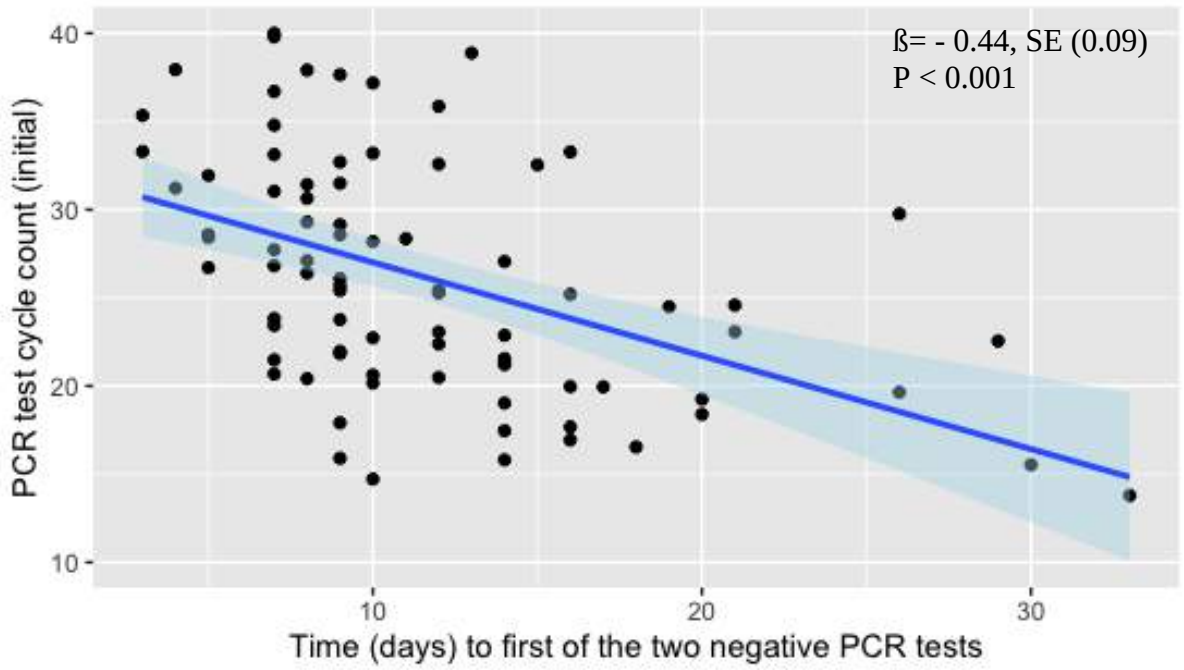

Fig 2: Duration of positive SARS-CoV-2 RT-PCR test according to initial cycle threshold (Ct) value for ORF1ab gene

Discussion:

Nearly one out of seven participants had a RT-PCR detected SARS-CoV-2 infection at the testing site of the Wahgnion mine in Burkina Faso. This suggest that miners could represent an important reservoir of SARS-CoV-2 transmission into the general population. Indeed, miners regularly travel to their original residency when they are not on duty and adequately identifying and isolating positive patients would be crucial to break the chain of transmission either in the general population or in the mine, which could help lower the impact of the disease on the miners productivity (12).

The prevalence of SARS-CoV-2 infection in this study was higher than that reported in the overall population (with prevalence of < 1%) and could accentuate the gradient of SARS-CoV-2 transmission from the mine to the general population (13). Much higher figures were reported in the country among high risk population including person with recent travel history from high risk countries, contact cases of COVID-19, clinically suspected cases and healthcare workers (5). Therefore, continuous surveillance is needed to timely identify transmission reservoirs, areas and persons most at risk for targeted preventive interventions. In this study, most of positive cases were from the nearby cities of the mining site, particularly among the local populations and suggest that population close to mining sites are more at risk, and need continuous surveillance and adequate preventive interventions.

Higher prevalence of SARS-COV-2 infection (although not statistically significant) was detected using the nasopharyngeal swabs compared to oropharyngeal swabs in our study. Many studies have reported that nasopharyngeal swabs may be more suitable than oropharyngeal swabs for the detection of SARS-CoV-2 infection (14–18). This suggest that routine surveillance of SARS-CoV-2 infection could primarily use nasopharyngeal swabs to increase the odds of detection. The RT-PCR assay is considered the 'gold standard' for the qualitative and quantitative molecular detection of SARS-CoV-2 nucleic acids (6). The technique has the advantage of test sensitivity of 95% (19), with a detection limit below 10 copies/reaction which allows early detection of low viral titers (20).

Initial cycle threshold (Ct) values are used to discriminate positive from negative samples, and we hypothesized that the initial Ct values could well correlate with the duration of the disease and thus, guide the frequency of the control testing and the return to daily activities (8). We also anticipate that adequately interpreting the initial Ct values could potentially help to predict the duration of the viral RNA shedding, and thus the possible duration of infectivity of COVID-19 patients. In this study, we found a significant correlation between initial viral load,

estimated by the initial RT-PCR test Ct values, and the duration of positive RT-PCR test results for both SARS-CoV-2 N and ORF1ab genes. Indeed, initial Ct values negatively correlated with the duration of the illness, indicating that individuals with an increased initial Ct value or lower viral load rapidly cleared their SARS-CoV-2 infection as compared to those with lower Ct values or higher viral load. For example, 42 (89.4%) of the 47 participants with Ct value over 25 could clear the infection within 14 days, while only 14 (40%) of the 35 participants with Ct value below 25 could clear the viral RNA within the same period. In the meantime, a total of 22% participants could not clear the viral RNA within the same period irrespective of the initial Ct values. Several studies have reported that RT-PCR Ct values strongly correlated with cultivable virus and the probability of culturing virus declines to 8% in samples with Ct >35 and 6% 10 days after onset of the disease irrespective of the presence of symptoms (21,22). Therefore, Ct values, could be used as criteria to approximate the duration of quarantine, given virus shedding is not associated with the presence of clinical symptoms (22).

Indeed, using the presence of clinical symptoms as a surrogate for decision making for daily activity return could lead to a higher risk of contamination at the work place, as their presence do not correlate with the infectivity period (22), and thus Ct values would play an important role for decision making. A study already reported a significant correlation between initial RT-PCR Ct values and the duration of the disease, however, their estimates could not be more precise due to their small sample size (11). Indeed, in this cited study, the average reduction time was 1.3 days per additional cycle ($\beta = -1.29 \pm 0.26$) using a sample of 25 patients, with larger standard errors. Our current study provided more accurate estimates, as higher sample size was used for the modeling, and this finding supports the use of Ct value to predict the duration of the illness, and thus allows a more efficient use of resource in low-income settings particularly the sub-Saharan Africa where infrastructure and qualified personnel are limited.

The knowledge of the relationship between Ct values and the duration of the disease can be used for targeted measure to reduce risk of transmission. In a recent study, longitudinal assessment of RT- PCR test results in individuals requiring 15–30 days to clear SARS-CoV-2 RNA showed that groups with initial high viral load (Ct values ≤25) and intermediate viral load (Ct values 26–30) exhibited a significant reduction of viral load between 8 and 14 days, and thus concluded that in patient with longer duration of disease, they may be of reduced infectivity after 14 days of isolation, however, the study could not confirm the absence of transmission beyond that period. Although we hypothesize from our study that the infectivity would be lower or even absent in high Ct values patients, we recommend that minimum prevention measures should be adopted by patients even after their return to daily activities.

Conclusion:

Our finding indicates that SARS-CoV-2 load estimates by Ct values on RT-PCR assay can be used to predict the duration of COVID-19 and infer the period of virus transmission. As participants with lower initial Ct values tended to have longer disease duration, initial RT-PCR Ct value could be used as a criterion to guide the return to work and daily activities.

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Contributions of authors:

The study was conceived by ZT. Data were collected by ZT, and AC. Data analysis was done by ML. The manuscript was drafted by ML. AC and ZT made important contributions to the final manuscript. All authors read and approved the final manuscript.

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Conflicts of interest:

Authors declare no conflict of interest.

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Original Article **Open Access**

Quality of life of people living with HIV and AIDS attending Irrua Specialist Teaching Hospital, Edo State, Nigeria

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Abstract:

Background: With the use of highly active antiretroviral therapy (HAART), life expectancy of HIV-infected persons had increased and the disease is now managed as a chronic one, but the quality of life (QOL) of the patients is now a concern. Social support enhances QOL of patients with chronic illnesses. However, no study has been done to determine the QOL of people living with HIV and AIDS (PLWHA) in our environment. This study therefore assessed the QOL of PLWHA attending antiretroviral therapy (ART) clinic of Irrua Specialist Teaching Hospital (ISTH), Edo State of Nigeria
Methodology: A descriptive cross-sectional study design was used. Two hundred and thirty PLWHA attending the ART clinic of ISTH, Irrua, Edo State, Nigeria, were systematically selected for the study. A structured questionnaire was interviewer-administered to collect data on sociodemographic and clinical profiles of selected participants, and the WHOQOL-HIV BREF questionnaire was used to collect data the QOL of each participant. Data were analyzed with IBM SPSS version 20.0.
Results: The overall mean QOL score for the participants was 89.13±1.18 (95% CI=87.95-90.31). The scores in three of the six life domains in the WHOQOL-HIV BREF instrument were similar and high; spirituality/ religion/personal beliefs (16.33±0.36), physical health (15.83±0.28) and psychological health (15.07±0.24). Lower mean QOL scores were observed in the social relationships (13.49±0.28) and environment (13.45±0.20) domains. Clinical HIV stage, marital status, educational status and gender were significantly associated with mean QOL scores in bivariate analysis while only HIV stage 1 and 2 were significantly associated with good QOL in multivariate logistic regression analysis.
Conclusion: It is pertinent that PLWHA are kept in early stages of HIV disease through combination of efforts such as prompt enrolment, commencement and monitoring compliance of HAART, and treatment of opportunistic infections, as well as public health measures including education, de-stigmatization, early diagnosis by extensive accessible screening/testing of at-risk population, social supports and economic empowerment, psychotherapy and social integration of affected individuals especially in a functional home.

Keywords: PLWHA, Quality of Life; HIV, HAART

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Qualité de vie des personnes vivant avec le VIH et le SIDA fréquentant Hôpital Universitaire Spécialisé d'Irrua, État d'Edo, Nigeria

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Résumé:

Contexte: Avec l'utilisation de la thérapie antirétrovirale hautement active (HAART), l'espérance de vie des personnes infectées par le VIH a augmenté et la maladie est désormais gérée comme une maladie chronique, mais la qualité de vie (QOL) des patients est désormais une préoccupation. Le soutien social améliore la qualité de vie des patients atteints de maladies chroniques. Cependant, aucune étude n'a été réalisée pour déterminer la qualité de vie des personnes vivant avec le VIH et le SIDA (PVVIH) dans notre environnement. Cette étude a donc évalué la qualité de vie des PVVIH fréquentant la clinique de thérapie antirétrovirale (ART) de l'hôpital d'enseignement spécialisé d'Irrua (ISTH), État d'Edo au Nigeria.
Méthodologie: Une conception d'étude transversale descriptive a été utilisée. Deux cent trente PVVIH fréquentant la clinique ART de l'ISTH, Irrua, État d'Edo, Nigeria, ont été systématiquement sélectionnées pour l'étude. Un questionnaire structuré a été administré par un intervieweur pour collecter des données sur les profils sociodémographiques et cliniques des participants sélectionnés, et le questionnaire WHOQOL-HIV BREF a été utilisé pour collecter des données sur la qualité de vie de chaque participant. Les données ont été analysées avec IBM SPSS version 20.0.
Résultats: Le score moyen global de qualité de vie des participants était de 89,13±1,18 (IC à 95%=87,95-90,31). Les scores dans trois des six domaines de la vie de l'instrument WHOQOL-HIV BREF étaient similaires et élevés ; spiritualité/religion/croyances personnelles (16,33±0,36), santé physique (15,83±0,28) et santé psychologique (15,07±0,24). Des scores moyens de qualité de vie inférieurs ont été observés dans les domaines des relations sociales (13,49±0,28) et de l'environnement (13,45±0,20). Le stade clinique du VIH, l'état matrimonial, le statut éducatif et le sexe étaient significativement associés aux scores moyens de qualité de vie dans l'analyse bivariée, tandis que seuls les stades 1 et 2 du VIH étaient significativement associés à une bonne qualité de vie dans l'analyse de régression logistique multivariée.
Conclusion: Il est pertinent que les PVVIH soient maintenues aux premiers stades de la maladie à VIH grâce à une combinaison d'efforts tels que l'inscription rapide, le début et le suivi de l'observance du HAART et le traitement des infections opportunistes, ainsi que des mesures de santé publique telles que l'éducation, la déstigmatisation, un diagnostic précoce par un dépistage/test accessible étendu de la population à risque, des soutiens sociaux et l'autonomisation économique, la psychothérapie et l'intégration sociale des personnes touchées, en particulier dans un foyer fonctionnel.

Mots clés: PVVIH, Qualité de Vie ; VIH, HAART

Introduction:

The development of highly active antiretroviral therapy (HAART) has shifted the perception of HIV/AIDS from an acute, fatal disease to a chronic and potentially manageable one (1). Antiretroviral therapy (ART) is capable of improving survival, reducing the occurrence of HIV-related opportunistic infections, improving patients clinical state and social capacity (1,2). Although HAART does not cure HIV disease, it significantly reduces the viral load and slows the progression of the disease, thereby increasing life expectancy of infected persons (2). Therefore, management of chronic debilitating diseases like HIV should aim not only at terminating disease progression and preventing death, but also at restoring or enhancing good quality of life (QOL) of affected individuals.

According to the World Health Organization (WHO), QOL is defined as "*individuals' perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, standards, expectations and concerns*" (3). There are a lot of advances in the medical management of HIV but the holistic support systems and QOL of these patients are often overlooked (4). The unwillingness to disclose HIV-serostatus due to associated social stigma and discrimination which in turn reduce health-seeking behaviour and adherent to clinic attendance are still major problems compounding the QOL of PLWHA (5). Also, the side effects of HAART, if present can also affect the QOL of an individual, with the knowledge that the drugs must be taken throughout the lifetime of the individual (1). Therefore, assessment of QOL aiming to provide a comprehensive evaluation of the individual well-being, including an appraisal of their functional role within the family, community integration and personal adjustment to life circumstances is very paramount (6).

Quality of life is considered synonymous with health status, functional and psychological well-being, happiness with life, satisfaction of needs, and assessment of one's own life (3,7). There are six life domains identified in the assessment of QOL. These are physical health, psychological health, level of independence, social relationship, environment, and spiritual/religion/personal domains (3). When QOL is related only to physical well-being or to lack of diseases or symptoms, the other areas of an individual life such as work, family, social relationships or psychological wellbeing are not included (7).

From the early focus on only physical (medical) functioning, QOL has shifted towards a more holistic approach to including all aspects of individual life and wellbeing. Quality, rather than quantity of life, is of paramount

importance for the continuous integration of PLWHA into the society and maintenance of their roles and functions (8). Quality of life in PLWHA is influenced by satisfaction with social support and coping styles, and in chronic diseases like HIV, assessing QOL has become an integral part of follow-up as it provides valuable feedback about therapeutic interventions and are indispensable in cost effective analysis (9).

HIV/AIDS has such serious repercussions on psychological, social and physical well-being, that the assessment of QOL of PLWHA will help to measure the extent of re-integration into the society after the crises they face at initial diagnosis, thus enabling them to meet their daily responsibilities (10). Information such as this is important in the evaluation of the impact of the disease on health outcomes, the effect of intervention already on ground, as well as the planning of other interventions that will help to meet other unidentified needs of PLWHA and improve the QOL of Nigerians living with HIV and AIDS.

While there is abundance of information on QOL of PLWHA, the few studies done in Nigeria were conducted in urban settings. This is the first time that QOL of PLWHA at the ART clinic of the Irrua Specialist Teaching Hospital (ISTH) will be assessed, as no study on QOL has been conducted in the hospital since inception till date. As a result, this study will provide baseline information on the perceived QOL of PLWHA in this rural setting, which has among other effects, social implications on everyone, because a person's perception of wellbeing will determine the acquisition of habits related to tasks, employment and care of others. The aim of this study therefore is to assess the QOL of PLWHA who access care from the ART clinic of ISTH, and to identify factors affecting their QOL.

Materials and method:

Study setting and design

This study is a descriptive cross-sectional design conducted at the ART clinic of ISTH Irrua, Edo State, south-south geopolitical region of Nigeria which has the highest prevalence of HIV/AIDS (3.1%) in Nigeria (11).

Study population and participants:

The study population were PLWHA attending the ART clinic of ISTH Irrua for treatment. Two hundred and thirty participants who have been on HAART for at least 12 months were recruited from the study population by systematic random sampling method. The criteria for exclusion were pregnancy and presence of co-morbidities such as diabetes mellitus, depression, HIV-encephalopathy, obesity, seizure disorders and hypertension.

Sample size and sampling technique:

The minimum sample size for the study was determined using the formula for proportion; $n=z^2pq/d^2$ (12), where 'n' is the desired sample size, 'z' is the normal standard deviation for the required level of confidence (1.96), 'p' is the estimated prevalence of 83% or 0.83 from a previous study (13), 'q' is 1-p and 'd' is the tolerable margin of error (set at 5% or 0.05). This gave a calculated sample size of 216, which was adjusted (based on 5% attrition rate) to 228, and rounded up to 230 HIV-infected adults as sample size for the study.

Eligible participants were consecutively recruited by systematic random sampling till the sample size was obtained. To calculate the k-value (i. e. sampling interval), the total number of adult PLWHA seen during the period of the study was divided by the sample size. The average number seen per month is 480, therefore, for a study that spanned over 3 months, the k-value was calculated as (480x3)/230 or 6.26. This made every 6^{th} adult participant who meets the eligibility criteria to be recruited for the study. The first participant was selected using a simple random sampling with ballot of numbers one to six. The second participant was randomly selected and subsequent participants were selected at interval of six. No participant was selected twice.

Ethical consideration:

Ethical approval was obtained from the Ethical Review Committee of ISTH, Irrua and informed consent was obtained from each participant.

Data collection:

Data collection was done using a two-sectioned structured-questionnaire, administered on consenting PLWHA who met the inclusion criteria for the study. The section A consisted of information regarding socio-demographic and clinical characteristics such as age, sex (gender), marital status, occupation, education level, place of residence, date of first enrolment, CD4 cell count, HIV status of partner, status disclosure and WHO clinical stage of participants. Section B contained a structured questionnaire for assessing QOL using the WHOQOL-HIV BREF instrument (14). WHOQOL-HIV BREF is a shorter version of WHOQOL-HIV instrument, which explores six domains of the QOL and a total of 31 questions for respondents to rate themselves on their QOL during the two weeks preceding the interview. Ans-

wers 1 to 5 were presented on a Likert scale, where 1 indicated low or negative perception and 5 indicated high or positive perceptions.

The six life domains identified in the WHOQOL-BREF instrument are; (i) physical health domain, which measures pain and discomfort, energy and fatigue, sleep and rest, with 4 questions (Q3,Q4,Q14,Q21); (ii) psychological health domain, which measures positive feelings, thinking, learning, memory and concentration, self-esteem, bodily image and appearances, and negative feelings, with 5 questions (Q6,Q11,Q15,Q24, Q31); (iii) level of independence domain which measures mobility and daily life activities, dependence on medications or treatments, and work capacity, with 4 questions (Q5,Q20,Q22,Q23); (iv) social relationship domain including personal relationships, social support and sexual activity, with 4 questions (Q17,Q25,Q26,Q27); (v) environment domain, which measures physical safety and security, home environment, financial resources, health and social care, accessibility and quality, opportunities for acquiring new information and skills, participation in and opportunities for recreation and leisure activities, and physical environment, with 8 questions (Q12, Q13,Q16,Q18,Q19,Q28,Q29,Q30); and (vi) spiritual/religion/personal beliefs domain, which measures forgiveness and blame, concerns about the future, and death and dying, with 4 questions (Q7,Q8,Q9,Q10). There was a general question on QOL, and another question on health in general, giving a total of 31 questions.

The score of the domains was obtained by multiplying the average score of the questions in the domain by 4, resulting in scores ranging from 4-20. The overall QOL score was obtained by the summation of the transformed scores from the six domains, resulting in scores ranging from 24-120. The higher the scores obtained, the better the QOL. Based on the Likert's scale, the QOL for each domain and the overall was categorized into five groups as shown in Table 1.

Statistical analysis of data:

After appropriate verification, the data were transferred into a master sheet and analyzed using IBM SPSS 20.0 software. Data collected from the study questionnaire were entered using numerical codes. Frequency distribution tables of variables were generated. Statistical comparison of variables was done using the Chi-squared test for categorical variables, and Students' '*t*' test (comparison of two means) and analysis of variance (comparison of more than 2 means) for continuous variables. A *p*-value of <0.05 was considered statistically significant.

Results:

Sociodemographic characteristics of study participants:

The sociodemographic characteristics of the participants are as shown in Table 2 below. The ages range of the participants was 20 to 68 years, and the mean age is 41.13±9.96 years.

Table 1: Quality of life categories based on transformed scores

S/N	Category	Domain transformed score	Overall transformed score
1	Very poor	4.00	24.00
2	Poor	4.01 - 8.00	24.01 - 48.00
3	Neither poor nor good	8.01 - 12.00	48.01 - 72.00
4	Good	12.01 - 16.00	72.01 - 96.00
5	Very good	16.01 - 20.00	96.01 - 120.00

Table 2: Sociodemographic characteristics of PLWHA in Irrua Specialist Teaching Hospital, Edo State, Nigeria

Variables	Frequency	Percent
Age group (in years)		
20-29	25	10.9
30-39	83	36.1
40-49	69	30.0
50-59	45	19.6
60-69	8	3.4
Age range (years)	20-68	
Mean age (±SD)	41.13 ± 9.96	
Age group (in years)		
15-45 (reproductive age group)	159	69.1
46-64 (middle age group)	69	30.0
65 (elderly)	2	0.9
Marital status		
Married	138	60.0
Widow/widower	47	20.4
Single	26	11.3
Separated	14	6.1
Cohabiter	3	1.3
Divorced	2	0.9
Gender		
Male	62	27.0
Female	168	73.0
Highest educational level		
No formal education	13	5.7
Primary	75	32.6
Secondary	79	34.3
Tertiary	63	27.4
Occupation		
Artisan	23	9.9
Civil servants	28	12.2
Driving	4	1.7
Farming	29	12.6
Students	10	4.3
Tailoring	7	2.2
Teaching	5	3.0
Trading	97	42.4
*Others	12	5.2
Unemployed	15	6.5
Religion		
Christianity	218	94.7
Islam	12	5.3
Traditional/others	0	0

*Others=Banker 1, Caterer 1, Clergy 1, Engineering 2, Industrialist 1, Journalist 1, Nurse 1, Policeman 2, Retired 2

Clinical profiles of the participants:

The clinical profiles of the participants are as shown in Table 3. Using the WHO clinical staging of the participants, 174 (69.6%) participants were in stage 1, 32 (13.9%) in stage 2, 14 (6.1%) in stage 3, and 10 (4.3%) in stage 4. The CD4 cell count of 160 (69.6%) participants was ≤ 500 cells/mm^3 while count for the remaining 70 (30.4%) was >500 cells/mm^3. As regards duration on HAART, 171 (74.4%) participants had been on the ART drugs for 1-5 years, 58 (25.2%) for 6-10 years, while only 1 (0.4%) had been on the drug for up to 11 years.

The body mass index (BMI) was normal for 135 (58.7%) participants (18.5-24.9 kg/m^2), 82 (35.6%) participants were overweight (BMI 25.0-29.9 kg/m^2), while 13 (5.7%) were underweight (BMI < 18.5 kg/m^2). Two hundred and twenty-four participants (97.39%) had good adherence to antiretrovirals, while only 6 (2.61%) had poor adherence to medications (Table 3).

Table 3: Clinical profile of people living with HIV/AIDS in Irrua Specialist Teaching Hospital, Edo State, Nigeria

Variables	Frequency	Percent
CD4 count (in mm^3)		
≤500	160	69.6
>500	70	30.4
WHO HIV staging		
Stage 1	174	75.7
Stage 2	32	13.9
Stage 3	14	6.1
Stage 4	10	4.3
Duration on HAART		
1-5 years	171	74.4
6-10 years	58	25.2
≥11 years	1	0.4
BMI		
Underweight <18.5kg/m^2	13	5.7
Normal (18.5 to 24.9kg/m^2)	135	58.7
Overweight (25.0 to 29.9kg/m^2	82	35.6
Adherence to HAART		
Good	224	97.39
Poor	6	2.61

BMI=Body mass index; HAART=Highly active ant-retroviral therapy; HIV= Human immunodeficiency virus; AIDS=Acquired immune deficiency syndrome; CD=Cluster of differentiation; WHO=World Health Organization

Table 4: Mean quality of life scores of PLWHA in Irrua Specialist Teaching Hospital, Edo State, Nigeria

Variables	Mean (± SE) quality of life scores	95% CI
Physical Health Domain	15.83±0.14	15.55 - 16.11
Psychological Health Domain	15.07±0.12	14.93 - 15.29
Level of Independence Domain	14.95±0.13	14.69 - 15.21
Social Relationship Domain	13.49±0.14	13.21 - 13.76
Environment Domain	13.45±0.10	13.25 - 13.65
Spiritual/Religion/Personal Beliefs Domain	16.33±0.18	15.97 - 16.69
Overall	**89.13±0.59**	**87.95 - 90.31**

SE=standard error; CI = Confidence interval

Mean quality of life scores of participants:

Table 4 shows the mean QOL scores of participants for each domain and the overall QOL score. The mean QOL score for; (i) physical domain at 95% confidence interval (CI) was 15.83±0.28; (ii) psychological domain 15.07±0.24; (iii) level of independence domain 14.95±0.26; (iv) social relationship domain 13.49±0.28; (v) environment domain 13.45± 0.20; and (vi) spiritual/religion/personal beliefs domain 16.33 ± 0.36. The overall QOL score was 89.13±1.18.

Association of socio-demographic factors with quality of life among participants:

The difference in the mean QOL scores with respect to the age of participants was statistically significant only in the social domain, with participants in the reproductive age group (15-45 years) having the highest mean QOL score of 13.71±2.29 (p=0.04) (Table 5). Male participants had higher overall QOL scores and mean QOL scores in all the domains than their female counterparts. The overall mean QOL score of 91.75±8.39 for males was significantly higher than 88.16±8.90 for females (t=7.496; p=0.007). The mean QOL score was significantly higher for males (p<0.05) in 4 of the 6 domains (psychological, level of independence, social and environment).

Concerning marital status of the participants, co-habiters, married participants and divorcees had significantly higher overall mean QOL scores (91.93 ± 9.41, 90.87 ± 8.90 and 90.30 ± 7.64 respectively), than the mean QOL

scores of separated, single and widowed participants (84.75±8.31, 84.75±8.32, and 88.12± 8.93 respectively) (F=4.033, *p*=0.002) (Table 5). However, there was no statistically significant difference in the overall mean QOL scores of employed (89.14±8.86) and unemployed participants (88.95±10.20) (*t*=0.01; *p*=0.937), and no significant difference in any of the six domains (*p*>0.05).

With respect to education, participants with no formal education had the highest overall QOL (96.42±4.65), followed by those without secondary education (89.87±8.67) and the least was those with primary school education (85.74±9.55). Those with tertiary education had the highest QOL scores in all the domains except in the social relationship and spirituality/religion/belief domains, where those with secondary and no formal education had the highest scores respectively (Table 5). Although, the participants who practice Islamic religion had a higher overall mean QOL scores (91.7± 10.17) than those who practice Christianity (88.93±8.86), the difference was not statistically significant (*t*=1.05, *p*=0.307), and there was also no significant difference in the QOL scores for the participants in all the domains with respect to religion (*p*>0.05).

Table 5: Association of sociodemographic factors with quality of life of PLWHA in ISTH Irrua, Edo State, Nigeria

Variables	Q0	Q1	Q2	Q3	Q4	Q5	Q6
Age group (years)							
15-45							
46-64	89.52±8.55	15.15±2.15	14.98±1.98	14.58±1.22	13.71±2.29	13.47±1.25	16.01±1.77
≥65	88.96±9.68	15.75±2.32	14.86±1.99	14.86±1.68	13.09±2.09	13.38±1.84	16.90±2.24
	84.96±16.33	15.00±3.53	15.02±1.03	13.50±3.53	11.50±2.12	14.25±0.35	15.00±5.66
F	0.44	0.90	0.108	229	3.262	4.000	0.671
P	0.0643	0.103	0.898	0.796	0.040*	0.671	0.092
Gender							
Male	91.75±8.39	16.22±1.87	15.56±1.83	15.50±1.64	14.01±2.51	13.76±1.47	16.73±2.50
Female	88.16±8.90	15.70±2.30	14.72±1.99	14.92±1.88	13.29±2.01	13.34±1.42	16.19±2.71
t	7.49	2.42	8.40	4.57	5.10	4.037	1.84
p	0.007*	0.12	0.004*	0.034*	0.025*	0.046*	0.176
Marital status							
Cohabiting	91.93±9.41	16.00±3.61	16.27±2.31	16.33±2.08	13.33±1.15	14.00±0.50	16.00±2.54
Divorced	90.30±7.64	17.50±3.54	16.80±3.39	15.60±0.00	9.00±2.83	15.50±4.24	15.50±2.12
Married	90.87±8.90	16.26±2.05	15.22±2.03	15.82±1.83	14.02±2.02	13.53±1.46	16.50±2.73
Separated	85.20±8.15	15.43±1.95	14.23±1.64	14.64±1.59	11.57±2.06	12.75±1.29	17.07±1.86
Single	84.75±8.31	15.76±1.88	15.38±1.40	15.61±1.47	13.08±2.43	13.86±0.96	15.50±3.24
Widow/Widower	88.12±8.93	14.68±2.39	13.95±1.79	14.04±1.92	12.91±1.87	13.07±1.41	16.33±2.66
F	4.033	4.22	4.43	0.14	7.24	2.82	0.93
p	0.002*	0.001*	0.001*	0.984	0.0001*	0.017*	0.465
Employment status							
Employed	89.14±8.86	15.86±2.19	14.98±1.95	14.95±2.23	13.47±2.17	13.42±1.46	16.36±2.63
Unemployed	88.95±10.20	15.47±2.42	14.54±2.35	15.46±2.24	13.73±2.31	13.90±1.18	15.93±3.12
t	0.01	0.46	1.01	0.07	0.21	1.54	0.36
p	0.937	0.499	0.317	0.795	0.651	0.216	0.547
Educational status							
None	96.42±4.65	16.31±1.75	15.20±1.03	15.23±1.54	13.15±1.63	13.15±1.55	17.38±1.76
Primary	85.73±9.55	15.18±2.53	14.40±2.25	14.46±2.07	12.72±2.17	13.06±1.68	15.86±2.93
Secondary	89.87±8.67	16.00±1.99	15.14±1.81	15.15±1.66	14.01±2.12	13.47±1.40	16.10±2.75
Tertiary	89.13±8.92	16.31±1.96	15.31±1.88	15.65±1.61	13.48±2.18	13.96±1.07	16.96±2.20
F	6.49	3.64	3.02	4.91	5.5	4.83	2.91
p	0.001*	0.013*	0.031*	0.003*	0.001*	0.035*	0.0001*
Religion							
Christianity	88.93±8.86	15.82±2.19	14.91±1.96	14.58±2.11	13.47±2.17	13.45±1.44	16.28±2.67
Islam	91.70±10.17	16.16±2.44	15.53±1.92	15.33±1.92	13.83±2.41	13.50±1.58	17.33±2.38
t	1.05	0.28	1.11	0.05	0.32	0.01	1.79
p	0.307	0.598	0.294	0.817	0.572	0.908	0.182

QO=Mean (±SD) Quality of Life Overall Scores; Q1=Mean (±SD) of Physical Health Domain Score; Q2=Mean (±SD) of Psychological Health Domain Score; Q3=Mean (±SD) Level of Independence Domain Score; Q4=Mean (±SD) of Social Relationship Domain Score; Q5=Mean (±SD) of Environment Domain Score; Q6=Mean (±SD) of Spiritual/Religion/Personal Beliefs Domain Score; F=ANOVA; *t*=Student t test; p=likelihood/probability; *=statistically significant

Association of HIV management care with quality of life of the participants:

The overall mean QOL scores of those who disclosed their HIV status to their partners (89.84±9.65) and those who concealed it from their partners (89.61±8.37), were not significantly different (t=1.05, p=0.308), and also across all the domains. The overall mean QOL scores of concordant partners, discordant partners and undefined group were not significantly different (F=0.16, p=0.850), and across all the domains. Irrespective of the CD4 counts, all the participants had similar QOL scores in all the domains, therefore CD4 counts had no significant impact on the QOL of the participants.

The overall QOL scores of participants decreases as the WHO clinical stage increases. Participants in WHO stage 1 had the highest QOL scores (90.36±7.61), followed by those in WHO stage 2 (88.34 ± 8.53), while those in stage 4 had the least QOL score (76.73±5.71) (F=9.94, p=0.0001). The pattern is similar in all the 6 domains (p<0.05). Irrespective of the duration on HAART, QOL scores of all the participants were similar across the domains. There was no statistically significant impact of length of time for HAART on the QOL among the participants (p>0.05) (Table 6).

Table 6: Association of HIV management care with quality of life of PLWHA in ISTH Irrua, Edo State, Nigeria

Variables	Q0	Q1	Q2	Q3	Q4	Q5	Q6
HIV status disclosure							
Yes	89.84±9.65	15.94±2.88	15.01±2.29	15.17±2.01	13.46±1.46	13.44±1.45	16.51±2.75
No	89.61±8.37	15.77±2.11	14.90±1.73	15.79±2.14	13.43±1.45	13.47±1.44	16.21±2.66
t	1.05	0.32	0.19	0.71	3.04	0.02	0.72
P	0.308	0.568	0.660	0.400	0.083	0.876	0.398
HIV status of partner							
Positive	89.40±9.95	15.88±2.36	14.85±2.41	15.09±2.07	13.71±2.07	13.35±1.42	16.51±2.80
Negative	89.27±8.61	15.79±2.12	15.15±2.07	14.98±2.07	13.43±2.41	13.55±1.57	16.03±2.93
Unknown	88.58±8.03	15.85±2.13	14.78±1.70	14.91±1.87	13.27±1.98	13.31±1.28	16.40±2.02
F	0.16	0.04	076	84	0.74	1.31	0.88
p	0.850	0.966	0.469	0.433	0.477	0.273	0.561
CD4 count (mm³)							
≤500	88.46±9.70	15.71±2.34	14.86±2.20	15.05±1.22	13.33±2.28	13.39±1.56	16.18±2.83
>500	90.66±6.67	16.21±1.83	15.15±1.35	15.25±1.53	13.84±1.88	13.58±1.14	16.68±2.19
t	2.97	1.74	1.09	0.43	2.71	0.85	1.76
P	0.086	0.188	0.298	0.513	0.101	1.756	0.786
HIV stage							
Stage 1	90.36±7.61	16.10±1.94	12.48±2.88	14.77±1.47	13.72±2.10	13.52±1.34	16.60±2.48
Stage 2	88.34±8.53	15.62±2.25	13.88±2.44	15.09±1.69	13.00±2.28	13.56±1.44	15.94±2.82
Stage 3	84.44±11.17	14.64±3.03	15.13±2.14	14.21±2.29	13.07±1.07	13.79±1.37	14.86±3.01
Stage 4	76.73±5.71	13.60±3.27	15.14±1.73	12.60±3.02	11.50±2.51	11.45±1.91	15.10±3.73
F	9.94	6.15	7.83	0.11	4.35	7.41	3.04
p	0.0001*	0.0001*	0.0001*	0.0001*	0.005*	0.0001*	0.030*
Duration on HAART (years)							
<1	86.37±10.56	15.29±1.99	15.00±2.35	14.58±2.09	12.58±2.92	13.55±1.62	15.35±3.06
1-5	89.32±8.75	15.93±2.13	15.07±1.87	15.03±3.12	13.53±2.19	13.42±1.46	16.23±2.73
6-10	89.35±8.98	15.75±2.45	14.60±2.14	15.03±1.96	13.63±1.87	13.48±1.36	16.84±2.27
≥11	89.12±8.39	15.84±2.02	14.94±1.98	13.00±0.08	13.00±0.07	15.00±0.17	19.00±0.14
F	0.643	0.753	0.077	0.165	1.099	0.433	3.04
p	0.588	0.522	0.508	0.920	0.350	0.729	0.030*

QO=Mean (±SD) Quality of Life Overall Scores; Q1=Mean (±SD) of Physical Health Domain Score; Q2=Mean (±SD) of Psychological Health Domain Score; Q3=Mean (±SD) Level of Independence Domain Score; Q4=Mean (±SD) of Social Relationship Domain Score; Q5=Mean (±SD) of Environment Domain Score; Q6=Mean (±SD) of Spiritual/Religion/Personal Beliefs Domain Score; HAART=Highly active ant-retroviral therapy; HIV=Human immunodeficiency virus; AIDS=Acquired immune deficiency syndrome; CD=Cluster of differentiation; F=ANOVA; *t*=Student t test; p=likelihood/probability; *=statistically significant

Table 7: Multivariate logistic regression analysis of participants characteristics with quality of life

Characteristics	β value	Odds ratio (95% CI)	*p* value
Gender			
Female	Reference group		
Male	2.64	14.070 (0.440 - 447.800)	0.134
Marital status			
Married	Reference group		
Single	-15.01	0.000 (0.000-)	0.999
Widow	-17.10	0.000 (0.000-)	1.000
Divorced	-0.74	0.480 (0.055-4.142)	0.503
Separated	-0.79	2.210 (0.057-85.36)	0.670
Cohabiting	-0.20	1.230 (0.086-17.43)	0.880
Educational status			
No formal education	Reference group		
Primary	-18.01	0.00 (0.00-)	0.999
Secondary	-0.85	2.33 (0.15-36.02)	0.554
Tertiary	1.66	5.25 (0.33-81.73)	0.236
WHO HIV staging			
Stage 1	Reference group		
Stage 2	-4.99	0.007 (0.001-0.075)	0.0001*
Stage 3	-3.79	0.023 (0.001-0.402)	0.01*
Stage 4	-2.26	0.105 (0.007-1.484)	0.095

Cox Snell R^2=11.7%; Negelkerke R^2=41.5%; Chi-squared=3.048; df=3; *p*=0.931;
CI=confidence interval; *=statistically significant

Multivariate logistic regression analysis of participant characteristics on quality of life:

The characteristics of the participants significantly associated with QOL scores on ANOVA and the Students' '*t*' tests i. e. gender, marital status, educational status and WHO staging were entered into multivariate logistic regression analysis model. Only WHO stage 1 and stage 2 were significantly associated with QOL on this model. The β value, represents the slope of the regression line, the variables bearing negative values, indicate that the higher the value of these variables, the higher the likelihood of having poor QOL (Table 7).

Discussion:

The introduction of HAART has led to increased live expectancy of PLWHA such that HIV infection is now being managed as a chronic disease and the QOL of the patients is now a significant consideration. Evidences are in support of improved QOL of patients with chronic illnesses including HIV/AIDS patients when there is adequate comprehensive healthcare addressing physical, social and psychological needs of affected individuals (8,15). This study investigated the QOL of PLWHA attending ART clinic in ISTH with focus on factors affecting QOL among these patients in a rural setting.

Of the 230 participants who met the inclusion criteria and were recruited into our study, 168 (73.0%) were females while 62 (27.0%) were males. This gender distribution is a reflection of the population of the clinic attendees, similar to the gender distribution in other studies reported by Fatiregun et al., (7) in Kogi State, Samson-Akpan et al., (16) in the southern senatorial district of Cross River State, and Azuka (17) in Benin-City, Edo State, Nigeria (17). This may be attributed to that fact that females have better health-seeking behaviour than males or have more time to attend clinics, while men are struggling to meet economic needs of the family (18,19,20).

The age range of the participants was 20-68 years, with a mean age of 41.13±9.96 years. Sixty-nine-point one percent (69.1%) of the participants were within the reproductive age group which has been confirmed in the literatures to be the age group with highest prevalence of HIV/AIDS (21,22). Only 2 (0.9%) were elderly, probably because of the reduced life expectancy of HIV patients relative to the general population as a result of weaker immunity with age, higher susceptibility to opportunistic infection and social and psychological challenges of the disease (22,23,24).

Only 13 (5.7%) of the participants had no formal education. Majority of them had either primary or secondary education while 63 (27.4%) had tertiary education training. This is similar to educational distribution reported by Fatiregun et al., (7) although, there were more illiterate participants in their study, and also similar to findings of the studies of Azuka (16) and Samson-Akpan et al., (16). Most of the participants in our study were Christians (94.7%) probably because the study site is located in a Christian dominated Esan land of

Edo State, Nigeria. The few Muslims (5.3%) were from the Etsako area of the State, dominated by Islamic religion. Despite the skewed statistics, there was no statistically significant difference between the two groups; and religion has no impact on QOL of participants in this study.

All the participants had been on HAART for at least 12 months prior to recruitment for this study, in order to exclude the initial shock or depression associated with early period of being HIV-diagnosed and to allow for beneficial effects of HAART. Choosing this category of participants also eliminates the initial challenges associated with initiating patients on HAART (adverse drug reactions) before the patients psychologically and physically adjusted. Most of the participants were in WHO stage 1 and 2 of HIV classification. This may partly be due to the exclusion of those with co-morbidities, as the 3rd and 4th stages are associated with many HIV/AIDS associated comorbidities; and partly due to good comprehensive HIV treatment, care and support services being provided at the study site.

In this study, PLWHA had good overall mean QOL score (89.13±0.59). This is similar to the findings of other researchers within and outside Nigeria, (1,3,7,16,17) where PLWHA had good QOL. The good QOL score in this study can be attributed to the use of ART and good adherence in all the patients studied for at least 12 months as reported. Oguntibeju (1) in a review article and Sophie et al., (9) in a Cochrane study observed that, several studies reported a strong positive association between ART and improved quality of life in different domains among PLWHA in both developed and developing countries (1). In addition, the good QOL of participants in this study may be partly due to sufficient time for recovery from the initial shock, denial and depression associated with being diagnosed HIV seropositive. Regular clinic attendance for 12 months and above, also provides opportunities for listening to health talks and meeting other HIV positives clients, these experiences can foster hope and ultimately improve the QOL of these patients as was reported by Sushil Yadav in PLWHA in Nepal (25). In addition, similarity in the QOL of participants in the study and those studies done in urban center of Nigeria, revealed that, use HAART irrespective of location of the PLWHA is important in QOL improvement of the affected individuals.

The highest mean QOL score was seen in the spiritual/religion/personal beliefs domain, followed by the physical health domain and the psychological health domain. This is similar to the findings of other researchers, especially Fatiregun et al., (7), Folasire et al., (4) and Samson-Akpan et al., (16). The high mean QOL score in the spiritual/religion/personal beliefs domain may be due to increase God-seeking behaviour and piety associated with encounters which are irreversible like HIV/AIDS and ultimately can lead to death. Boppana and Gross (25) and Villani et al., (26) observed that significant number of their patients reported an increase in spirituality/religiosity post-HIV diagnosis, aside of Nigeria being a highly religious country (27). Religiosity can impact on psychic and physical well-being of patients.

The high mean QOL score in the physical domain can also be attributed to ART capability to improving survival, reducing the occurrence of HIV-related opportunistic infections, and improving patients' clinical state and thus physical wellbeing (25,26). The environment domain had the lowest mean QOL score followed by social relationship domain. This may be because PLWHA face various social and environmental problems, such as stigmatization, discrimination, poverty, and cultural beliefs, which can affect their QOL socially, leading to problems that affect important activities and interests of the persons (1) These findings are in consonance with those of Fatiregun et al., (7) study, which also highlighted low mean scores in environment domain. Similar results were also reported in Sao Paulo, Brazil (28), where the mean scores for social relationship and environment domains fell in the intermediate level.

Environment domain measures financial resources, freedom, physical safety and security, health and social care; accessibility and quality, home environment, opportunity for acquiring new information and skills, participation in and opportunities for recreation/leisure activities, physical environment (pollution/ noise/traffic/climate) and transport (13). The results indicating low scores for environment in this study may imply lack of money and poor living conditions since most of the respondents were of primary and secondary school level, living in rural communities of Edo State, whose earnings may not be adequate for personal and social needs. Being employed provides some level of financial independence and thus reduces some of the stresses on them. Good clinical stage in most of these participants may make their HIV status obscured except they disclosed it. This in turn reduces chances of stigmatization, discrimination and other psychosocial negative effects of HIV on their relationship with other family members.

In the bivariate analysis in this study,

only four factors had impact on the participants' QOL. These are gender, marital status, level of education and the WHO HIV clinical stage. These factors were among those identified by other researchers to influence QOL of HIV patients (1,7,9). Men had better QOL in all the domains and overall, except in the spiritual /religion/belief domain. The odds of a male having good QOL life is 14.07 times higher than a female having good QOL. This may not be unconnected to the superior socio-psychological position and stronger economic strength of men in the society relative to their female counterparts (29). Our study is at variance with the findings of Fatiregun et al., (7), who reported that women had higher QOL score compared to men in virtually all domains, and significantly higher level on the independent domain. The men had better QOL in our study, probably because they have equal access to all health-promoting factors such as HAART, care, and support, like the women, and extra advantage of being more empowered by tradition, and being less exposed to intimate partner violence and rejection than women. The findings on gender in our study also differs from the report of Samson-Akpan et al., (16) in southern senatorial district of Cross River State, where gender difference was reported to be insignificant. The disparities in the studies may due to some sociodemographic differences and methodology of the studies. For instance, the selection of participants in the Samson-Akpan et al., (16) study was based on purposive sampling on the basis of regular participation at group meetings, which may have biased participants selection. The higher QOL of women reported in their study is not surprising as women are known to be more religious and resort to faith, than men.

With regards to marital status of the participants as significant factor impacting on QOL in this study, cohabiters, married and divorcees had higher mean QOL scores than widows and singles in almost all the domains. The regression analysis showed very bad QOL prognosis for widow/widower and the singles. The β value representing the slope of the regression line for the variables bearing negative values, indicate that the higher the value of these variables the higher the likelihood of having poor quality of life. A stable relationship conceivably contributes to a good QOL. This was shown convincingly in almost all domains in this study and other studies (30,31). Conflicting results were found with respect to changes in QOL over time. In women, being single was associated with an improvement in QOL after four months, whereas being married was associated with a decline (9). Being chronically ill possibly causes more health distress and anxiety in married women, since the illness can interfere with their role as a spouse. However, a stable partner was also found to be positively associated with mental health after one year (31).

Among socioeconomic variables, having no formal education or having any level of education appeared to have effect on QOL in all domains. However, regression analysis revealed that level of education has no significant associated with QOL of PLWHA. Good education is expected to enhance acquisition of health promoting information on internets and other resource materials (31) and adherence to HAART (13,30) as well as other health promoting activities. It also often implies higher income and thus better living conditions and health (3). However, this study is at variance to other studies cited. This may be due to high level of awareness in the study area (32), as only 5.7% of the participants have no formal education. In Edo state, even an illiterate can communicate well in 'pigin-english', hence they can comprehend health education and clinical instructions very well.

The strongest factor determining good QOL is WHO clinical stage 1 or 2, which was the only variable with strong association on logistic regression model. An objective of HAART is to enhance the clinical stage and delay progression into late stages of the disease. A result other than this is tantamount to treatment failure if adherence is not compromised, since clinical failure lags behind virologic and immunologic failures. This findings is similar to reports of review and cochrane studies on QOL of PLWHA (1,9).

Conclusion:

Quality of life in PLWHA is influenced ultimately by just one factor; the WHO Clinical stage of the PLWHA. Good QOL is obtained and maintained by being in early WHO clinical stage i. e. stage 1 and 2. It is therefore very pertinent that PLWHA are kept in the early stages of the disease. This is achievable by combination of efforts and actions such as intense public awareness and public health education on HIV/AIDS, de-stigmatization of the disease and elimination of discrimination against PLWHA; early diagnosis by extensive accessible screening/testing of at risk population; prompt enrolment of HIV-seropositive persons into care; quick commencement of HAART with strong emphasis on adherence counseling; monitoring

adherence; managing side effects promptly and switching drugs when necessary; continuous regular clinical, immunologic and virologic monitoring and reevaluation of patients; as well as prompt diagnosis and treatment of opportunistic conditions associated with the disease. Furthermore, social supports, economic empowerment, psychotherapy and social integration of affected individuals, especially in a functional home will go a long way in enhancing the QOL.

Contributions of authors:

ABT conceived the idea and conceptualized the study, AGM coordinates and heads the HIV/AIDS program of the institution directly managing the patients. The two authors were involved in literature search, development of research proposal, data collection and analysis, and writing of manuscript for publication.

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There is no conflict of interest.

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Original Article **Open Access**

Polymerase chain reaction detection of haemolysin D gene (*hlyD*) in uropathogenic *Escherichia coli* as a novel diagnostic test for urinary tract infection

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Abstract:

Background: Urinary tract infection (UTI) is a common and sometime serious infectious disease diagnosed using conventional urine culture as the 'gold standard' for identifying *Escherichia coli,* the most common causative agent. However, due to the slow turn-around-time and other challenges of urine culture, this study explores the use of a novel biomolecular polymerase chain reaction (PCR) approach to detect the presence of haemolysin D gene (*hlyD*) that encodes a unique virulence factor of uropathogenic *E. coli* (UPEC) for its rapid identification in UTI.
Methodology: Primers from UPEC CFT073 and non-pathogenic *E. coli* K-12 MG1655 strains provided by Nottingham Trent University, England, UK were used to investigate the presence of haemolysin D gene (*hlyD*) in UPEC. The *hlyD* primers were developed from *hlyD* with locus number C_RS01660 on UPEC CFT073 strain using the NCBI, virulence finder, and Island viewer, and used in a PCR assay to target the *hlyD* in UPEC. Three sets of PCR templates were designed (UPEC, *E. coli,* and "No template"), each with internal and external controls amplified in a multiplex PCR assay, and agarose gel electrophoresis was used to separate the amplicons, and determine the specificity of *hlyD* for UPEC.
Results: The UPEC genome PCR assays were positive for *hlyD* and UPEC positive control, and similarly, PCR was positive for *E. coli* genome positive control, but negative for *hlyD*. Moreover, the "No template" PCR assay was clean with no amplification product, confirming the absence of PCR contaminations.
Conclusion: The *hlyD* is a unique virulence gene specific for UPEC. PCR assay of this gene is a promising specific and rapid biomolecular diagnostic test that can overcome the limitations of the traditional approaches for detection of UPEC in UTI.

Keywords: UTI, uropathogenic *Escherichia coli,* virulence factors, PCR, diagnosis

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Détection par réaction en chaîne de la polymérase du gène de l'hémolysine D (*hlyD*) dans *Escherichia coli* uropathogène comme nouveau test de diagnostic pour l'infection des voies urinaires

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Résumé:

Contexte: L'infection des voies urinaires (UTI) est une maladie infectieuse courante et parfois grave diagnostiquée à l'aide d'une culture d'urine conventionnelle comme «étalon-or» pour identifier *Escherichia coli*, l'agent causal le plus courant. Cependant, en raison du délai d'exécution lent et d'autres défis de la culture d'urine, cette étude explore l'utilisation d'une nouvelle approche biomoléculaire de réaction en chaîne par polymérase (PCR) pour détecter la présence du gène de l'hémolysine D (*hlyD*) qui code pour une virulence unique facteur d'*E. coli* uropathogène (UPEC) pour son identification rapide dans les infections urinaires.
Méthodologie: Des amorces provenant des souches UPEC CFT073 et non pathogènes d'*E. coli* K-12 MG1655 fournies par l'Université de Nottingham Trent, Angleterre, Royaume-Uni, ont été utilisées pour étudier la présence du gène de l'hémolysine D (*hlyD*) dans l'UPEC. Les amorces *hlyD* ont été développées à partir de *hlyD* avec le numéro de locus C_RS01660 sur la souche UPEC CFT073 à l'aide du NCBI, du détecteur de virulence et de la visionneuse d'île, et utilisées dans un test PCR pour cibler le *hlyD* dans UPEC. Trois ensembles de modèles de PCR ont été conçus (UPEC, *E. coli* et «aucun modèle»), chacun avec des contrôles internes et externes amplifiés dans un test de PCR multiplex, et une électrophorèse sur gel d'agarose a été utilisée pour séparer les amplicons et déterminer la spécificité de *hlyD* pour l'UPEC.
Résultats: Les tests PCR du génome UPEC étaient positifs pour *hlyD* et le contrôle positif UPEC, et de même, la PCR était positive pour le contrôle positif du génome *E. coli*, mais négative pour *hlyD*. De plus, le test PCR "sans matrice" était propre sans produit d'amplification, confirmant l'absence de contaminations par PCR.
Conclusion: Le *hlyD* est un gène de virulence unique spécifique de l'UPEC. Le test PCR de ce gène est un test de diagnostic biomoléculaire spécifique et rapide prometteur qui peut surmonter les limites des approches traditionnelles de détection de l'UPEC dans les infections urinaires.

Mots clés: IVU, *Escherichia coli* uropathogène, facteurs de virulence, PCR, diagnostic

Introduction:

Urinary tract infection (UTI) is a crucial health issue, affecting almost 175 million people globally (1). Particularly in women, UTI is the most frequent infectious disease often associated with severe outcomes (2). A spectrum of UTI infection spans from asymptomatic to symptomatic bacteriuria, and acute UTI to chronic forms that can lead to serious conditions such as pyelonephritis and cystitis (1). Severe UTI is usually recurrent, difficult-to-treat, and may be fatal (3). Approximately $3 billion is spent each year on UTI management in the United States healthcare system (1).

Uropathogenic *Escherichia coli* (UPEC) remains the main etiologic agent of uncomplicated UTI accounting for at least 80% incidence (2,4). A similar statistic was reported in the US regarding community-acquired UTI, and UPEC usually coexist with *Staphylococcus saprophyticus*, a Gram-positive agent of UTI with a prevalence of 10-15%, and often have a similar presentation among humans, with associated bacteriuria (1). Rapid identification of UPEC is crucial for patient management and quick epidemiological investigations.

Escherichia coli, a member of the family *Enterobacteriaceae*, is a gastrointestinal tract symbiont, but many pathogenic *E. coli* strains with varying virulence genes can cause severe infections (5,6). UPEC is a common extraintestinal pathogenic *E. coli* (ExPEC) that causes community-acquired UTI associated with morbidity and mortality globally due to the large range of associated virulence genes (5,7). The ExPEC is also known for its carriage of many virulence genes in pathogenicity islands (5) such as toxins (*hlyA, cnf*, and auto-transporter), iron acquisition and transport systems, adhesins, invasins, lipopolysaccharides, proteins, and siderophores. These factors equipped the pathogen with the ability to colonize and invade the urinary tract through the blood stream, and also empower ExPEC with antibiotic resistance (3,8). The UPEC virulence factors are two-fold; secreted toxins and factors found on the bacterial cell surface (5). Secreted *E. coli* toxins facilitate illness through inflammation activation (5).

Haemolysin D (*hlyD*) is an essential constituent of typical alpha-haemolysin (*hlyA*) along with *hlyB* and *tolC*, forming a transporter complex through which *hlyA* can be directly moved to the extracellular medium (5). Furthermore, haemolysin production confers *E. coli* with higher pathogenicity and persistence in the host, leading to recurrence of extra-intestinal infections (9,10). Exploring the relevant virulence/pathogenic genes versus the strain type is of great importance for diagnosis, therapy, and vaccine development for pathogenic *E. coli* (11).

Laboratory approaches for the diagnosis UTI include urine dipstick, culture, and microscopic urinalysis (12). At present, the standard urine culture is the 'gold standard' test, being highly reliable (95% sensitivity and 85% specificity), and although it is able to differentiate *E. coli* from other uropathogens, the turnaround time is prolonged, making it unsuitable for prompt therapy of uncomplicated UTI. Other setbacks of urine cultures include failure to grow atypical microbes and false-positive culture results from contamination (13). Rapid UTI kits have a high false-positive rate, inaccuracies, and low specificity, even though more promising modalities are being investigated (14). Contrastingly, the flow-cytometer-based urine analyzer can scr-

een out two-thirds of samples that did not require standard urine culture, thereby decreasing the urine culture workload (15).

The limitations encountered in the traditional biochemical testing methods of *E. coli* give way to molecular diagnostic techniques such as the polymerase chain reaction (PCR) assay (2). PCR assay is now the most successfully recognized technique for the detection of nucleic acids (2), targeting different genes of specific uropathogens (1). It is used for rapid identification of specific bacterial pathogens through the use of primers for several targeted genes in one reaction tube cutting down on turnaround time and costs (13). This study aims to determine the specificity of the *hlyD* virulence gene in UPEC as a diagnostic target in PCR assay.

Materials and method:

Study setting:

The study was conducted in 2017 as part of the Master of Science (MSc) course works of the corresponding author in Biomedical Science at the Microbiology Laboratory of Nottingham Trent University, England, United Kingdom

Bacteria strains and primer designs:

UPEC CFT073 and K-12 MG1655 strains (provided by Nottingham Trent University) were used in this study as PCR genome templates for UPEC (positive and internal controls) and *E. coli* primers respectively (Table 1). The extraction, purification and PCR assay of the bacterial DNA to obtain these PCR templates were done using GeneXpert system (Cepheid, USA), and made available to the main author by the module tutor (Dr Jonathan Thomas). These were used to investigate the presence of *hlyD* as a virulence gene in UPEC.

The unique *hlyD* virulence gene was first identified in UPEC as a diagnostic target using PCR assay, bioinformatic and comparative genome analyses. The *hlyD* with locus number (C_RS01660) on UPEC CFT073 and gene sequence (Table 1) was chosen and provided as a (UPEC primer) unique virulence gene, which was selected using the National Center for Biotechnology Information (NCBI), virulence finder, and Island viewer, in order to examine its presence in UPEC and *E. coli* by PCR assay in this study.

UPEC PCRs set up:

Master-mix was prepared in an Eppendorf tube to contain 35µl Mango Mix, 26µl water, and 3µl of UPEC CFT073 gDNA; 17µl of this master-mix was pipetted into each of the three labelled PCR tubes (UPEC pTest, UPEC pUPEC, and UPEC pEc) and then 1.5µl of each 10µM primer (left and right primers, control UPEC-F and control UPEC-R, and control Ec-F and control Ec-R) were also added respectively.

Non-pathogenic *E. coli* PCRs set up:

Master-mix was prepared in an Eppendorf tube to contain 35µl Mango Mix, 26 µl of water, and 3µl of non-pathogenic *E. coli* MG 1655 gDNA; 17µl of this master mix was pipetted into each of the three labeled PCR tubes (Ec pTest, Ec pUPEC, and Ec pEc), and then 1.5µl of each 10µM primer (left and right primers, control UPEC-F and control UPEC-R, and control Ec-F and control Ec-R) were also added respectively.

No template control PCRs set up:

"No template" control PCR master mix was prepared in an Eppendorf tube to contain 35µl Mango Mix and 26µl water; 17µl of this master-mix was pipetted into each of the three labeled PCR tubes (NTC pTest, NTC pUPEC, and NTC pEc), and then 1.5µl of each 10µM primer (left and right primers, control UPEC-F and control UPEC-R, and control Ec-F and control Ec-R) were also added respectively.

PCR amplification:

The amplification of the virulence gene was done in a PCR machine (Eppendorf Master Cycler). The nine labeled PCR reaction tubes (3 from each UPEC, non-pathogenic *E. coli*, and "No template" control) were placed in the PCR machine and ran under the following conditions; 1 cycle of 95°C for 90 secs, 35 cycles of 95°C for 30 secs, 52°C for 60 secs, 72°C for 90 secs, and 1 cycle of 72°C for 5 min.

Agarose gel electrophoresis of PCR amplicons:

Electrophoresis of the PCR products was done by pipetting 10µl of each of the nine prepared PCR reactions into the corresponding labelled wells of the agarose gel alongside 5µl of 1kb DNA ladder used as a molecular size marker. The agarose gel was placed in an electrophoretic machine and ran for 45 minutes under an electrical current at 120v (16,17). The DNA amplicons were visualized in the gel under ultraviolet transilluminator as bands following ethidium bromide staining (18).

Results:

Fig 1 shows gel electrophoresis picture of the PCR amplicons of *hlyD* primers in UPEC CFT-073 and *E. coli* K-12MG1655 strains. For UPEC genomic PCR, the result is *hlyD* positive as the test result in lane 1 is positive with a size amplicon of ≥ 300 base pairs (bp). Also, *E. coli* control (K-12 MG1655) PCR in lane 2 is positive with a big size amplicon of almost more than 700 bp, and UPEC control (CFT073) PCR in lane 3 was very weak with primer-

dimer of less than 300 bp (weakly positive).

For *E. coli* genomic PCR, the result is *hlyD* negative as the test result in lane 4 appears with no DNA band, and the UPEC control (CFT073) PCR in lane 6 is also negative with no products, only the *E. coli* control (K-12 MG 1655) PCR in lane 5 showed positive DNA band of almost more than 700 bp. For the "No template" PCR, the lanes were clean with no products, indicating that the PCR preparations were good with no contamination.

Table 1: DNA sequences of primers designed for the study

Genome/gene	Forward sequence/Reverse sequence (F/R)	Length	T_m (°C)	GC%
UPEC (CFT073)	F- 5'-GTGGCAGTATGAGTAATGACCGTTA-3'	25	56	44.0
	R- 5'-ATATCCTTTCTGCAGGGATGCAATA-3'	25	54	40.0
E. coli (K-12 MG1655)	F- 5'-ATGGAAAGTAAAGTAGTTGTTCCGGCACA-3'	29	59	41.4
	R- 5'-GGACGCAGCAGGATCTGTT-3'	19	53	57.9
Haemolysin D (*hly D*)	F 5'-GGTTCAGCGAGTTCCTGTTG-3'	20	54	55.0
	R 5'-TACCGAGTTTGTTCCAGCCT-3'	20	52	50.0

T_m = annealing temperature; GC = Guanine-cytosine content; UPEC = uropathogenic *Escherichia coli*

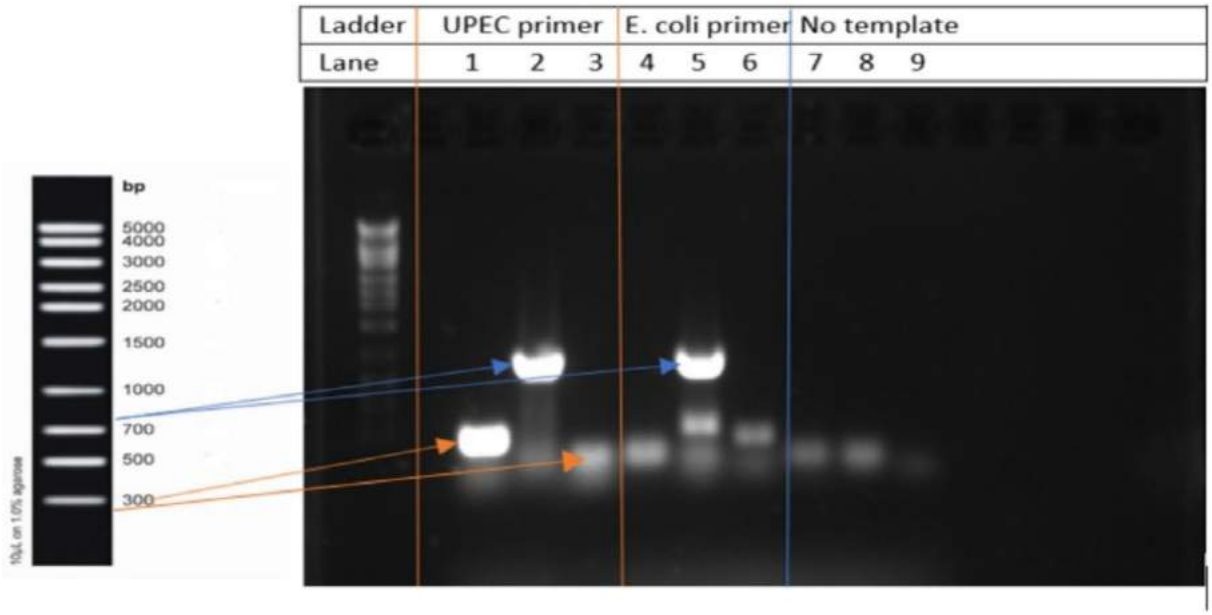

Fig 1: Gel electrophoresis of the PCR products observed through ultraviolet transillumination

The first column is a molecular ladder lane, the second column are UPEC genomic amplicons, the third column are *E. coli* genomic amplicons, and the fourth are "No template" amplicons. Lane 1 is UPEC test (*hlyD*) positive amplicon, lane 2 is *E. coli* control amplicon, lane 3 is UPEC weak positive control amplicon (with primer-dimer), lane 4 is *E. coli* test (*hlyD*) negative amplicon, lane 5 is *E. coli* control amplicon, lane 6 is UPEC negative control amplicon, and lanes 7, 8, and 9, are "No template" negative amplicons.

Discussion:

Simple urinary tract infection is a common condition, almost 50% of women globally will have an incidence of at least an episode of UTI in their lifetime. As a frequent health challenge, it can result in reduced productivity, poor ill health, and enormous economic costs each year (4). The haemolysin D virulence factor is of particular importance in UPEC colonization of the urinary tract and induction of major inflammatory pathways such as NF-κβ through TLR-4 signaling (5). Rapid identification of UPEC in UTI is therefore crucial for patient management and quick epidemiological investigations.

In this study, the designed *hlyD* primers detected only UPEC and not *E. coli* genomes, which implies that primers for *hlyD* are specific only for UPEC. In addition, for a valid result, the UPEC positive control primers should only detect UPEC and not *E. coli* genomes. In this study, UPEC positive control primers detected only UPEC genomes though with a very weak expected amplicon size of nearly 300 bp, but negative in the case of *E. coli* with no detectable amplicon product. This validated that the PCR conditions were optimal (primers, UPEC genomic DNA, and deoxynucleotide triphosphates). However, in the case of *E. coli* control primers, the primers can detect any type of *E. coli* genomes. Therefore, in this study, the *E. coli* control primers detected both the UPEC genome (lane 2) and the *E. coli* genome (lane 5) all with greater than or equal to 700bp amplicon size. There was no amplicon in the template control PCRs in this study, indicating the absence of contamination in both the PCR preparations and reactions (19).

The notable finding of this study is the fact that PCR could differentiate UPEC from non-pathogenic *E. coli* by detecting the specific *hlyD* virulence gene. Vysakh and colleagues (20) reported similar findings in their multiplex PCR assays using *hlyD*, *papC*, and *sfa* genes to target biofilm-forming rapidly growing UPEC (strain BRL-17) in a urine sample of patients with severe UTI.

Conclusion:

Diagnostic PCR technique performed in this study was simple and specific for the detection of *hlyD* in UPEC strain. Accordingly, this approach can serve as alternative to the traditional urine culture for the detection UPEC in UTI. However, there is the need for further investigations of this assay directly on urine samples to confirm this result.

Contributions of authors:

ANH conceptualize the study and performed the laboratory analysis; ANH, AM, and JM perform literature search and wrote the initial draft; ANH, MY, JM, and AM wrote the final draft; AM, JM and MY performed critical revision of the manuscript; and ANH and AM revised the manuscript. All authors agreed to the final manuscript submitted.

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Conflict of interest:

No conflict of interest is declared

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Original Article **Open Access**

Resistance profiles of urinary *Escherichia coli* and *Klebsiella pneumoniae* isolates to antibiotics commonly prescribed for treatment of urinary tract infections at Monkole Hospital Center, Kinshasa, Democratic Republic of the Congo

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Abstract:

Background: The occurrence of urinary tract infection (UTI) caused by multi-drug resistant bacteria is increasing worldwide and has become a major public health concern that requires global attention. To promote better treatment outcome of UTI and raise awareness of antibiotic resistance in the Democratic Republic of the Congo (DRC), we investigated the antimicrobial resistance profile of bacterial pathogens frequently isolated from urine samples of inpatients and outpatients with symptoms of UTI at the Monkole Hospital Center (MHC), Kinshasa from June 2017 to May 2018.
Methodology: This was a retrospective review of results of uro-cultures of urine samples of both inpatients and outpatients who had clinical symptoms of UTI, over a period of one year at the MHC, Kinshasa, DRC. During this period, aerobic uro-cultures of urine were done on MacConkey agar (MAC) or Cystine-Lactose-Electrolyte-Deficient (CLED) agar media at 37°C incubation for 24 hours. Identification of bacterial isolates on the culture media and antimicrobial susceptibility to sixteen selected antibiotics were done using the integral system enterobacteria and the Vitek® 2 automated system according to the manufacturer's instructions. The R-studio software was used for statistical analysis.
Results: Of the 2765 uro-cultures performed during the period of study, 809 (29.3%) were positive for bacteria with *Escherichia coli* being the most frequently isolated bacteria pathogens. There was no significant difference (p>0.05) in the resistance rates of both *E. coli* and *Klebsiella pneumoniae* to most of the antibiotics such as amoxicillin-clavulanic acid, piperacillin-tazobactam, amikacin, levofloxacin, norfloxacin, cefuroxime, cefotaxime, cefixime and cephalexin but resistance rates of *E. coli* compared to *K. pneumoniae* was significantly higher to cotrimoxazole (OR=2.06, p=0.0016), ofloxacin (OR=3.43, p=0.0019), ciprofloxacin (OR=1.624, p=0.044) and significantly lower to imipenem (OR=0.037, p=0.0046), nitrofurantoin (OR=0.292, p=0.0004) and fosfomycin (OR=0.311, p=0.0003). Both pathogens showed resistance rates of more than 50.0% to doxycycline, cefuroxime, cefixime and cephalexin but resistance rates of *K. pneumoniae* to ofloxacin and cotrimoxazole was less than 50.0%. The isolates were least resistant to imipenem, piperacillin-tazobactam and amikacin, with less than 13.0% resistance rate.
Conclusion: The findings of this study showed that *E. coli* is the most isolated bacterial uro-pathogen amongst patients with UTI at MHC Kinshasa, DRC, but both *E. coli* and *K. pneumoniae* were resistant to most commonly prescribed antibiotics used for treatment of UTI. Amikacin, piperacillin-tazobactam and imipenem demonstrated high *invitro* activity and should be prioritized for antimicrobial stewardship to prevent or delay emergence of resistance to them. To guarantee optimal treatment of UTI, regular pathogen surveillance and local antibiogram

reporting are required. Further studies are needed in DRC to assess the burden and factors driving antimicrobial resistance nationwide.

Keywords: urinary tract infection, bacteria; susceptibility; resistance; profile

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Profils de résistance des isolats urinaires d'*Escherichia coli* et de *Klebsiella pneumoniae* aux antibiotiques couramment prescrits pour le traitement des infections des voies urinaires au Centre Hospitalier Monkole, Kinshasa, République Démocratique du Congo

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Résumé:

Contexte: La survenue d'infections des voies urinaires (IVU) causées par des bactéries multirésistantes est en augmentation dans le monde et est devenue un problème majeur de santé publique qui nécessite une attention mondiale. Pour promouvoir de meilleurs résultats de traitement des infections urinaires et sensibiliser à la résistance aux antibiotiques en République démocratique du Congo (RDC), nous avons étudié le profil de résistance aux antimicrobiens des agents pathogènes bactériens fréquemment isolés à partir d'échantillons d'urine de patients hospitalisés et ambulatoires présentant des symptômes d'infection urinaire à l'hôpital de Monkole. (MHC), Kinshasa de juin 2017 à mai 2018.
Méthodologie: Il s'agissait d'une revue rétrospective des résultats d'urocultures d'échantillons d'urine de patients hospitalisés et ambulatoires qui présentaient des symptômes cliniques d'IU, sur une période d'un an au MHC, Kinshasa, RDC. Au cours de cette période, des urocultures aérobies d'urine ont été réalisées sur de la gélose MacConkey (MAC) ou de la gélose Cystine-Lactose-Electrolyte-Deficient (CLED) à 37°C d'incubation pendant 24 heures. L'identification des isolats bactériens sur les milieux de culture et la sensibilité antimicro-bienne à seize antibiotiques sélectionnés ont été effectuées à l'aide du système intégral d'entérobactéries et du système automatisé Vitek® 2 conformément aux instructions du fabricant. Le logiciel R-studio a été utilisé pour l'analyse statistique.
Résultats: Sur les 2765 urocultures réalisées au cours de la période d'étude, 809 (29,3%) étaient positives pour les bactéries, *Escherichia coli* étant la bactérie pathogène la plus fréquemment isolée. Il n'y avait pas de différence significative (p>0,05) dans les taux de résistance d'*E. coli* et de *Klebsiella pneumoniae* à la plupart des antibiotiques tels que l'amoxicilline-acide clavulanique, la pipéracilline-tazobactam, l'amikacine, la lévo-floxacine, la norfloxacine, la céfuroxime, la céfotaxime, la céfixime et céphalexine, mais les taux de résistance d'*E. coli* par rapport à *K. pneumoniae* étaient significativement plus élevés pour le cotrimoxazole (OR=2,06, p=0,0016), l'ofloxacine (OR=3,43, p=0,0019), la ciprofloxacine (OR=1,624, p=0,044) et significativement inférieur à l'imipénem (OR=0,037, p=0,0046), à la nitrofurantoïne (OR=0,292, p=0,0004) et à la fosfomycine (OR=0,311, p=0,0003). Les deux agents pathogènes ont montré des taux de résistance de plus de 50,0% à la doxycycline, au céfuroxime, au céfixime et à la céphalexine, mais les taux de résistance de *K. pneumoniae* à l'ofloxacine et au cotrimoxazole étaient inférieurs à 50,0%. Les isolats étaient les moins résistants à l'imipénème, à la pipéracilline-tazobactam et à l'amikacine, avec un taux de résistance inférieur à 13,0%.
Conclusion: Les résultats de cette étude ont montré qu'*E. coli* est l'uropathogène bactérien le plus isolé parmi les patients atteints d'infection urinaire au CMH de Kinshasa, en RDC, mais *E. coli* et *K. pneumoniae* étaient résistants aux antibiotiques les plus couramment prescrits utilisés pour le traitement des UTI. L'amikacine, la pipéracilline-tazobactam et l'imipénem ont démontré une activité in vitro élevée et devraient être prioritaires pour la gestion des antimicrobiens afin de prévenir ou de retarder l'émergence d'une résistance à ces derniers.

Pour garantir un traitement optimal des infections urinaires, une surveillance régulière des agents pathogènes et des rapports d'antibiogrammes locaux sont nécessaires. D'autres études sont nécessaires en RDC pour évaluer le fardeau et les facteurs à l'origine de la résistance aux antimicrobiens à l'échelle nationale.

Mots clés: infection urinaire; bactérie; susceptibilité; résistance; le profil

Introduction:

Urinary tract infections (UTIs) are one of the most common infectious diseases affecting approximately 150–250 million people worldwide every year (1). UTI refer to any type of urothelial inflammatory response that results from periurethral infection by one or several uropathogens usually residing in the gut (2). The periurethral infection can become invasive upon colonization of the urethra and pathogen migration to the bladder. The uropathogenic bacteria that invade the bladder can subsequently ascend to the kidneys and spread to the bloodstream to cause bacteraemia. Certain uropathogens such as *Escherichia coli* and *Pseudomonas aeruginosa* can also form biofilms leading to recurrent UTIs. Other serious complications include pyelonephritis with sepsis that can lead to renal damage and death (3).

Urinary tract infection profoundly affects the quality of life of affected individuals, who are predominantly females of all ages, infant boys, older men, and people with diabetes mellitus (3). It is estimated that 50% of females experience at least one UTI in their lifetime due to their short urethra that allows bacteria to easily reach the bladder from the anal region to cause ascending infection. This also applies to female children whose anus are usually wiped towards the genital area by most parents during body hygiene, which increase the risk of UTI in young girls (1). The urinary retention caused by uterine atony during pregnancy and at menopause may also contribute to bacterial proliferation in the urine (4). UTI result in considerable public health burdens, healthcare cost and societal costs such as time missed from work (3,5).

Antibiotics are currently the most recommended therapeutics for patients with UTI (5). These antimicrobials mostly belong to the classes of aminoglycoside, carbapenem, cephalosporin, penicillin and quinolone (6-8). However, antibiotic treatment of UTI is now challenged by the emergence and spread of multi-drug resistant (MDR) pathogens due to worldwide misuse of antibiotics (6-8). Limited access to quality, safe, efficacious, and affordable medical products also contribute to the emergence of antimicrobial resistance (AMR) in Democratic Republic of the Congo (DRC).

The mechanisms of resistance to antibiotics include inactivation of drug by microbial enzymes, enhanced drug efflux, limited uptake of drug, and alterations of the drug target (9). The presence of acquired plasmids encoding extended-spectrum β-lactamases (ESBLs) in some uropathogens such as *E. coli* and *K. pneumoniae* may also explain the rapid transmission of AMR pathogens in humans (3,10). Additionally, the emergence of class C β-lactamases (AmpC enzymes) and mobile-colistin-resistance (*mcr*) gene in certain bacterial strains is also associated with spread of multi-drug resistance (3,11).

These situations make the provision of appropriate antimicrobial therapies more challenging and the treatment of UTI more perplexing. This underlines the need for efficient strategies to promote better management of UTI, rational use of antibiotics and prevention of the emergence of MDR bacteria in our communities. Among these strategies, the determination of antibiotic sensitivity and resistance profiles of bacterial uropathogens is an affordable and easy way to constantly update the healthcare professionals about the status of antimicrobial susceptibility of urinary pathogens. The aim of this study therefore is to identify the most frequently encountered bacteria pathogens in urine samples of patients with clinical diagnosis of UTI at the Monkole Hospital Center, Kinshasa, DRC. In line with the World Health Organization (WHO) Global Action Plan (GAP) on AMR, the study also aimed to determine the resistance profile of these uropathogens to commonly used antibiotics as evidence for future guidance in UTI care in DRC.

Materials and method:

Study setting and population:

This study was conducted from June 1 2017 to May 31 2018 at the Monkole Hospital Center (MHC), a teaching hospital located in Mont-Ngafula, a township in Kinshasa Province in the western DRC. This healthcare facility is one of the health reference centers in Kinshasa, a city of more than 12 million inhabitants. The MHC provides medical care to approximately 100,000 people for both inpatient and outpatient annually. This study population consisted of both inpatients and outpatients with clinical suspicion of UTI.

Study design:

This study was a retrospective observational review of uro-cultures of patients with clinical symptoms and signs suggestive of UTI at MHC from June 2017 to May 2018.

Data were retrieved from the hospital registers on all patients with UTI irrespective of gender and age within the study period.

Ethical consideration:

The study was approved by the Medical and Directory Board of Monkole Health Center, Kinshasa, DRC. Data were carefully handled to avoid any link with the patient's identity to safeguard anonymity.

Specimen culture and bacteria identification:

During the period of study reviewed, urinary samples were aseptically collected in disposable sterile containers from both inpatients and outpatients including voided and catheter urine specimens. All the specimens were cultured within 30 minutes of collection in the biomedical laboratory of MHC on either MacConkey agar (MAC) or Cystine-Lactose-Electrolyte-Deficient (CLED) agar media (Becton Dickinson, Heidelberg, Germany). Urine samples were inoculated with calibrated platinum loop (0.01 ml) on the culture plates, and incubated aerobically at 37°C for 24 hours. Urine cultures with colonies count of ≥ 10^5 CFU/ml were considered indicative of UTI and were subjected to further analysis for bacterial pathogen identification.

For bacterial identification, colonies growing on culture plates were identified to species level using the integral system enterobacteria (Liofilchem, Roseto DA, Italy) and Vitek® 2 automated system (BioMérieux, Craponne, France) in line with manufacturer's instructions**.**

Antibiotic susceptibility test:

The antimicrobial susceptibility test was performed on each isolate using the integral system enterobacteria (Liofilchem, Roseto DA, Italy) and Vitek® 2 automated system (BioMérieux, Craponne, France) in line with the manufacturer's instructions. Sixteen antibiotics were used; imipenem, piperacillin-tazobactam, amikacin, nitrofurantoin, fosfomycin, levofloxacin, amoxicillin-clavulanic acid, ciprofloxacin, cefotaxime, norfloxacin, ofloxacin, doxycycline, cotrimoxazole, cefuroxime, cefixime and cephalexin.

Statistical analysis:

Statistical analysis of data was done using R studio. Data visualization was done with boxplot, interaction plot and ggline. Levene's test was used to assess homogeneity of variance and Shapiro-Wilk test to assess how close the data fit to a normal distribution. Two-way analysis of variance was used to test for significance of more than two means of continuous variables, and Pearson's Chi-squared test was used to compare the frequency of *E. coli* and *K. pneumoniae* and their antimicrobial resistance profiles in the analyzed samples. The significance level was $p<0.05$.

Results:

Results of data assessment:

The data assessment for linearity using Shapiro-Wilk test showed normally distributed population ($p>0.05$), and this was confirmed by the trend observed in the normal probability plot (Fig 1), in which almost all observations lie approximately on the straight line. The Levene's test for equality of variance of all observations confirmed that the variance is equal ($p>0.05$), hence the assumption check for the two-way ANOVA was met and the two-way ANOVA result is summarized in Table 1.

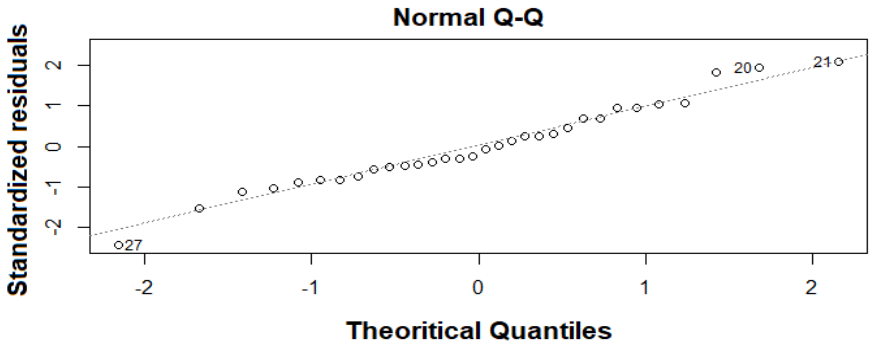

Fig 1: Normal probability plot

Table 1: Two-way analysis of variance

	Df	Sum Sq	Mean Sq	F value	p value
Antibiotic	1	13,970	13,970	188.633	5.79 e^{-14}
Pathogen	1	1	1	0.017	0.896
Antibiotic: Pathogen	1	45	45	0.602	0.444
Residuals	28	2,074	74		

Frequency of bacterial pathogens in UTI: During the 12-month study period, 2765 uro-cultures were reviewed and 809 (29.3%) were positive for significant bacterial pathogens with *E. coli* and *K. pneumoniae* the most prevalent (Table 2 and Fig 2). *Escherichia coli* was the single most frequently isolated bacterial pathogen (57.2%, n=463) followed by *K. pneumoniae* (14.8%, n=120) and other uropathogens (28.0%, n=226).

Of the total 809 isolates, 806 (99.6%) are Gram-negative bacteria belonging to 5 orders, 10 families, 18 genera and 35 species of bacteria (8 of which could not be identified to the exact species level) while 3 (0.4%) are Gram-positive belonging to 2 orders, 2 families, 2 genera and 3 species of bacteria (Table 2). The most frequently isolated species other than *E. coli* and *K. pneumoniae* are *Klebsiella oxytoca*, *Enterobacter cloacae, Citrobacter freundii, Serratia fonticola, Pseudomonas aeruginosa, Proteus mirabilis* and others, constituting 28% (n=226) of the uropathogens (Table 2).

Table 2: Frequency distribution of order, family, genera and species of bacterial uropathogens isolated from patients with urinary tract infection in Monkole Hospital Center, Kinshasa, Democratic Republic of the Congo

Order	Family	Genus	Species	Frequency
Gram negative				
Enterobacterales	Enterobacteriaceae	Escherichia	*Escherichia coli*	463 (57.2)
		Klebsiella	*Klebsiella pneumoniae*	120 (14.8)
			Klebsiella oxytoca	24 (3.0)
		Enterobacter	*Enterobacter cloacae*	43 (5.3)
			Enterobacter aerogenes	12 (1.5)
			Enterobacter hafnia	11 (1.4)
		Citrobacter	*Citrobacter* spp	39 (4.8)
			Citrobacter freundii	4 (0.5)
			Citrobacter koseri	1 (0.1)
		Arizona	*Arizona* spp	30 (3.7)
		Salmonella	*Salmonella* spp	1 (0.1)
		Raoultella	*Raoultnella ornithinolytica*	3 (0.4)
	Yersinaceae	Serratia	*Serratia fonticola*	4 (0.5)
			Serratia liquefaciens	4 (0.5)
			Serratia spp	9 (1.1)
			Serratia odorifera	1 (0.1)
			Serratia rubidaea	1 (0.1)
	Morganellaceae	Morganella	*Morganella morganii*	1 (0.1)
		Proteus	*Proteus mirabilis*	6 (0.7)
			Proteus vulgaris	2 (0.3)
		Providencia	*Providencia rettgeri*	2 (0.2)
			Providencia stuartii	1 (0.1)
	Hafniaceae	Edwardsiella	*Edwardsiella* spp	2 (0.3)
	Erwiniaceae	Pantoea	*Pantoea* spp	1 (0.1)
Pseudomonadales	Pseudomonadaceae	Pseudomonas	*Pseudomonas aeruginosa*	5 (0.6)
			Pseudomonas spp	3 (0.4)
			Pseudomonas oryzihabitans	2 (0.2)
			Pseudomonas paucimobilis	2 (0.2)
			Pseudomonas luteola	1 (0.1)
			Pseudomonas putida	1 (0.1)
	Moraxellaceae	Acinetobacter	*Acinetobacter baumannii*	3 (0.4)
			Acinetobacter lwoffii	1 (0.1)
Sphingomonadales	Sphinomonadaceae	Sphingomonas	*Sphingomonas paucimobilis*	2 (0.3)
Aeromonadales	Aeromonadaceae	Aeromonas	*Aeromonas* spp	1 (0.1)
Neisseriales	Neisseriaceae	Chromobacterium	*Chromobacterium violaceum*	1 (0.1)
Gram positive				
Bacillales	Staphylococcaceae	Staphylococcus	*Staphylococcus cohnii*	1 (0.1)
			Staphylococcus saprophyticus	1 (0.1)
Actinomycetales	Micrococcaceae	Kocuria	*Kocuria kristinae*	1 (0.1)
Total				**809 (100)**

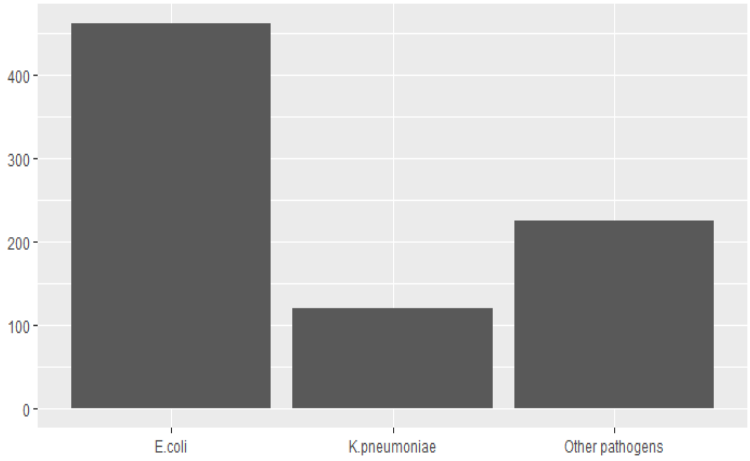

Fig 2: Frequency of isolation of bacterial pathogens from urine of patients with suspected urinary tract infection in Monkole Health Center, Kinshasa, DRC.

Resistance profiles of *E. coli* and *K. pneumoniae* isolates:

The resistance profile showed that both pathogens exhibited highest resistance to cephalexin while *E. coli* exhibited least resistance to imipenem (0%) and *K. pneumoniae* to piperacillin-tazobactam (5.1%) as shown Fig 3a, Fig 3b, Fig 4 and Table 3. Both *E. coli* and *K. pneumoniae* had similar trend in resistance rates (p>0.05) to most of the antibiotics; piperacillin-tazobactam, amikacin, levofloxacin, norfloxacin, amoxicillin-clavulanic acid, cefotaxime, cefuroxime, cefixime, cephalexin and doxycycline (Fig 3b, Table 3).

By bivariate analysis however, the resistance rates of *E. coli* compared to *K. pneumoniae* were significantly higher to cotrimoxazole (OR=2.060, p=0.002), ofloxacin (OR=3.427, p=0.0019), ciprofloxacin (OR= 1.624, p=0.0442), and significantly lower to imipenem (OR=0.03704, p=0.0049), nitrofurantoin (OR=0.2930, p=0.0004) and fosfomycin (OR=0.3112, p=0.0003) (Table 3).

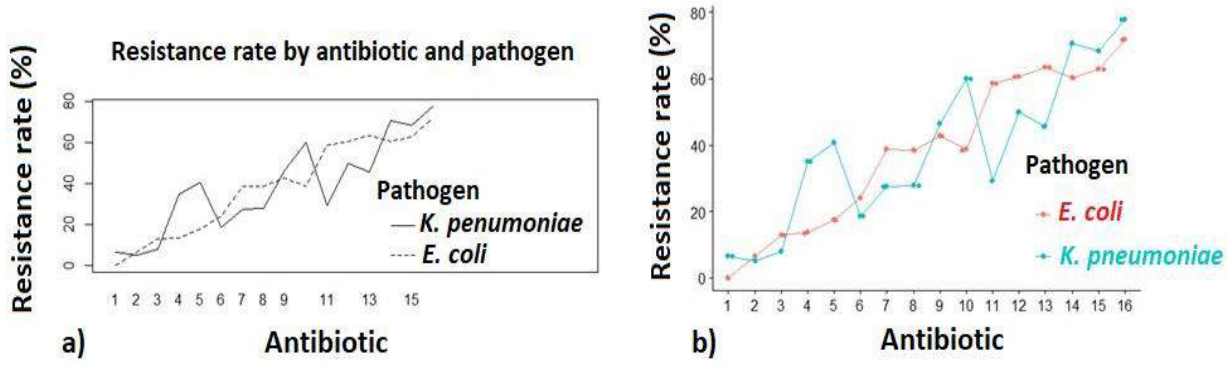

Fig 3. Interaction plot (a) and line plot (b) for resistance rate by antibiotic and pathogen. Antibiotics are codified from 1 to 16 as shown in Table 2.

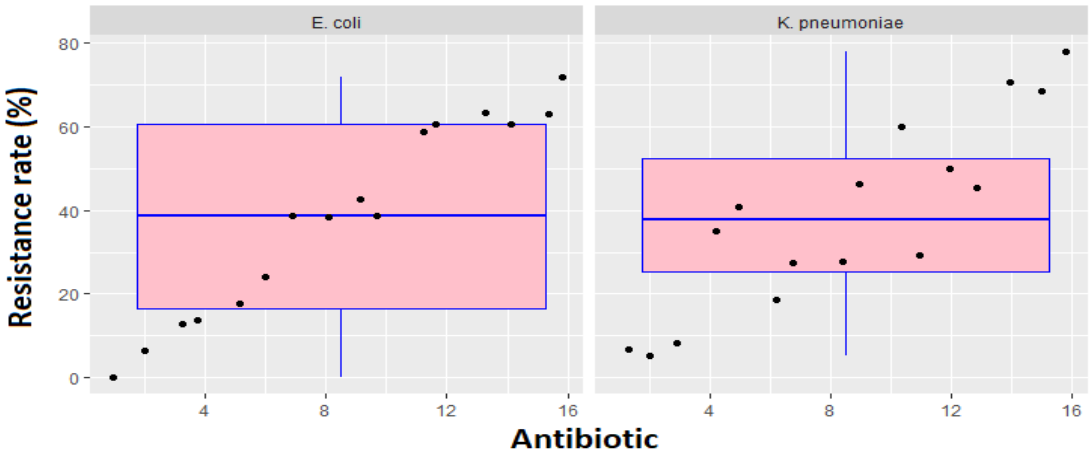

Fig 4: Boxplot distribution of resistance rate of *E. coli* and *K. pneumoniae* to antibiotics

Table 3: Comparative frequency of resistance of urinary *Escherichia coli* and *Klebsiella pneumoniae* isolates to sixteen selected antibiotics routinely used in Monkole Hospital Center, Kinshasa, Democratic Republic of the Congo

S/N	Antibiotic name	Percentage (number) Resistance to *Escherichia coli*	Percentage (number) Resistance to *Klebsiella pneumoniae*	x^2	COR (95% CI)	P value
1	Imipenem	0 (0/172)	6.6 (4/61)	7.918	0.03704 (0.00196-0699)	0.0049*
2	Piperacillin-Tazobactam	6.5 (26/402)	5.1 (5/99)	0.849	0.787 (0.345-1.795)	0.7707
3	Amikacin	13 (55/422)	8.1 (8/99)	1.414	1.705 (0.7842-3.706	0.2345
4	Nitrofurantoin	13.6 (25/184)	34.9 (22/63)	12.51	0.2930 (0.1502-0.5715	0.0004*
5	Fosfomycin	17.6 (49/278)	40.7 (22/54)	13.02	0.3112 (0.1667-0.5812)	0.0003*
6	Levofloxacin	24.2 (79/327)	18.7 (14/75)	0.749	1.389 (0.7364-2.616)	0.3868
7	Amoxicillin-Clavulanic acid	38.8 (59/152)	27.5 (14/51)	1.677	1.677 (0.8357-3.364)	0.1954
8	Ciprofloxacin	38.4 (168/437)	27.8 (30/108)	3.811	1.624 (1.022-2.580)	0.0442*
9	Cefotaxime	42.7 (194/454)	46.4 (52/112)	0.361	0.8609 (0.5683-1.304)	0.5482
10	Norfloxacin	38.6 (17/44)	60 (12/20)	1.744	0.4198 (0.1424-1.238)	0.1867
11	Ofloxacin	58.6 (78/133)	29.3 (12/41)	9.688	3.427 (1.609-7.301)	0.0019*
12	Doxycycline	60.5 (23/38)	50 (6/12)	0.095	1.533 (0.4156-5.658)	0.7576
13	Cotrimoxazole	63.3 (274/433)	45.5 (46/101)	10.00	2.060 (0.1330-3.192)	0.0016*
14	Cefuroxime	60.4 (32/53)	70.6 (12/17)	0.221	0.6349 (0.1951-2.066)	0.6385
15	Cefixime	63 (34/54)	68.4 (13/19)	0.022	0.7846 (0.2575-2.391)	0.8817
16	Cephalexin	71.8 (28/39)	77.8 (14/18)	0.235	0.7273 (0.1958-2.701)	0.8782

Numbers in brackets indicate the proportion of resistant isolates to total number of isolates tested per antibiotic; x^2=Chi square, COR=Crude Odd Ratio; CI=Confidence Interval; S/N = serial number of antibiotics; * = statistically significant at $p<0.05$

Discussion:

This study reports *E. coli* prevalence of 57.3% among bacterial pathogens isolated from patients with clinical UTI during the one-year period of study (June 2017 and May 2018) at Monkole Health Center in Kinshasa, Democratic Republic of the Congo. *Escherichia coli* is a commensal bacterium of the human intestinal microbiota, which is highly frequent in faecal samples, as reported recently in a study conducted among Congolese students (12). Due to the anatomic proximity of the anal zone to the genito-urinary area in humans, poor hygiene may justify the high incidence of *E. coli* in UTI as this bacterium can easily colonize the urinary tract and predispose to UTI (13).

The prevalence of *E. coli* in our study is comparable to the 58.5% reported in North Kivu province in the eastern part of DRC, and to the prevalence reported in previous studies from different countries worldwide (10, 14-19). However, a few studies have repor-

ted different rates where *E. coli* accounted for less than 20% of UTI cases (20,21). These differences suggest the existence of remarkable variability in the prevalence of uropathogens from one place to another. On the other hand, the prevalence of 14.8% reported for *K. pneumoniae* is within the range of 13-18% reported from studies conducted in the province of Kivu, DRC as well as from other countries such as Algeria, Bangladesh, Equatorial Guinea and Morocco (14,16,22,23), but higher than 2-9% rate reported from studies conducted in Honduras, Nigeria, China and Oman (10,15,17,24,25). Also, the prevalence of rare uropathogens in our study (~28%) agrees with the findings of a study conducted in Central Europe between 2011 and 2019 (14).

The urinary *E. coli* and *K. pneumoniae* isolates in our study showed high resistance to most of the first and second line oral and parenteral antibiotics such as cotrimoxazole, ofloxacin, ciprofloxacin, levofloxacin, amoxicillin-clavulanate and cefuroxime commonly used in Kinshasa, confirming the existence and challenges of AMR in bacterial uropathogens in DRC. Levofloxacin and nitrofurantoin remain the drug of choice among oral antibiotics, and have been advocated as first-line treatment of UTI in Kinshasa in line with international guidelines, yet the average resistance rate of *E. coli* and *K. pneumoniae* to both antibiotics in our study is over 20%, which is a warning sign of emerging resistance to them (26,27). Although norfloxacin and ofloxacin are recognized in several countries as effective fluoroquinolones against bacterial uropathogens, resistance rates in the present study varies from 30 to 60% for both *E. coli* and *K. pneumoniae,* which indicates that these antibiotics may not be effective in treatment of UTI in DRC.

The high resistance profile to antibiotics observed in this study can be accounted for by the overuse of these antibiotics leading to long-term exposure of both *E. coli* and *K. pneumoniae* to these antibiotics in Kinshasa. Indeed, most healthcare practitioners often prescribe one of these drugs for treatment of UTI, and sometimes without laboratory evidence of antibiotic susceptibility testing on these isolates. In addition to this, self-medication is a driving factor as this practice is associated with inappropriate use of antibiotics. Therefore, there is a need to reinforce control measures for antibiotic use and protect available antimicrobials from misuse in order prevent or delay emergence of resistance to them. This is the case for antibiotics such as imipenem, piperacillin-tazobactam and amikacin which can be proposed as second-line antibiotics for treatment of UTIs in Kinshasa, in line with the World Health Organization AWaRe categorization of antimicrobials (28). The observed low resistance rate of both pathogens to these antibiotics may be explained by the fact that they are not commonly prescribed in Kinshasa owing to their limited availability in open market.

Our findings should provide reliable information to assist practitioners who usually treat UTI empirically. Nevertheless, it should be noted that empirical antibiotic therapy based on epidemiological data does not always consider variations in susceptibility that may occur temporally and locally (or even regionally). This situation may lead to the emergence of MDR bacteria. Consequently, it will be important to emphasize antimicrobial therapy based on susceptibility patterns of pathogens. In this way, it will be possible to streamline the use of antibiotics and guarantee that they are as effective as possible in treatment of UTIs by using only molecules that demonstrate good results in susceptibility tests, thereby reducing the over exposure of bacteria to other antibiotics. Our findings should also set the stage for the establishment of a public health database for antimicrobial resistance surveillance as well as for the implementation of local policies for the rational use of antibiotics in DRC. Indeed, it is of great importance for hospitals and institutional care to know and update their local resistance profiles to antibiotics since empirical antibiotic therapy is based on epidemiological data. This strategy may reduce the medical costs as well as the morbidity and mortality rates caused by UTI. On the other hand, epidemiological databases may serve as a referential tool ensuring effective implementation of the policy adopted for UTIs management. They also make possible the identification of new points of intervention for controlling antimicrobial resistance.

In line with the above and considering the temporal and geographic variations in antimicrobial susceptibility, further investigations are needed nationwide to tackle the emergence and spread of antimicrobial resistance in DRC. In this context, public and professional education coupled with general awareness programs on the importance of good personal hygiene and environmental sanitation are of interest. Moreover, the promotion and evaluation of medical and veterinary practice guidelines constitute critical issues to tackle antimicrobial resistance. Excessive use of antibiotics in animal health and production has been identified among the driving forces of antimicrobial resistance (29,30). Therefore, in the context of "One Health", good antimicrobial stewardship at the hospital, in the animal sector as well as at the community level is crucial to slow down the emergence of antibiotic resistance.

In addition, curbing the spread of antibiotic-resistant pathogens in Kinshasa will also entail dealing with the promotion of affordable healthcare as well as fighting against substandard and counterfeit drugs.

We acknowledged some limitations of this study. For instance, the antimicrobial susceptibility testing of the uropathogens was simultaneously performed on both inpatients and outpatients, which makes it impossible to determine the susceptibility rates of bacteria to antibiotics in each group of patients. Moreover, the lack of distinction between community and hospital-acquired infections did not allow further investigations of the risk factors that may influence drug management and preventive measures. Being a retrospective review, there is the limitation of data accessibility and completeness, which did not allow us to include data beyond 2018 in the analysis. Therefore, future studies should focus on addressing these limitations and conducting cross-sectional or prospective cohort studies that can generate complete data.

Conclusion:

Our study showed that *E. coli* is the most frequent bacterial uropathogen isolated from patients with clinically suspected UTI at MHC, Kinshasa, DRC. We also observed that both *E. coli* and *K. pneumoniae* exhibited high resistance rates to the first and second-line antibiotics such as cotrimoxazole, levofloxacin, ofloxacin, ciprofloxacin, cefuroxime and cefotaxime used in DRC. Amikacin, piperacillin-tazobactam and imipenem were the most effective antibiotics with the pathogen demonstrating least resistance to them .

To guarantee optimal therapy of UTI, regular surveillance of AMR pathogens and generation of periodic antibiogram are required to guide empirical therapy. Further studies are needed to determine the national prevalence of nosocomial and community-acquired UTI and the antimicrobial resistance profiles of uropathogens. There is a dire need to develop the DRC national action plan to tackle AMR, which should be implemented following the "One Health' approach.

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Contributions of authors:

GKM was involved in study conceptualization, investigation, methodology, data collection, data analysis, resource provision, validation, visualization, writing the first manuscript draft, review and editing; FNL was involved in formal analysis, investigation, methodology, resource provision, validation, review and editing; OKK was involved in investigation, methodology, review and editing; JMIL was involved in investigation, methodology, review and editing; LMT was involved study conceptualization, formal analysis, methodology, project administration, validation, review and editing; PBM was involved in study conceptualization, formal analysis, resource provision, supervision, validation, review and editing, and fund acquisition.

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Conflict of interest:

Authors declare no conflict of interest

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Original Article **Open Access**

Antimicrobial properties, safety, and probiotic attributes of lactic acid bacteria isolated from Sauerkraut

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Abstract:

Background: According to the World Health Organization, probiotics have been defined as live microorganisms that when administered in the right amount provide health benefits to the host. This study aims to isolate lactic acid bacteria (LAB) from Sauerkraut and determine their anti-pathogenic potential and suitability as probiotics.
Methodology: Lactic acid bacteria (LAB) were isolated from fermented cabbage obtained from an open market in Akure, Ondo State, Nigeria by inoculating the prepared cabbage suspension on triplet plates of Rogosa agar (*Lactobacillus* selecting agar) and incubating in an anaerobic jar with gaspaks at 37°C for 72 hours. The LAB isolates were presumptively identified phenotypically by colony morphology, Gram stain reaction, and catalase test. The antibacterial activity of the LAB isolates was then carried out using agar overlay and agar-well diffusion methods. The most efficient LAB isolate was selected based on its strong antibacterial activity, confirmed by 16S rRNA sequencing and further evaluated for probiotic activities including bile salt resistance, survival in low pH, hydrophobicity of the cell surface, auto-aggregation, and co-aggregation. The putative probiotic LAB isolate was also evaluated for its safety using *in vitro* tests (antibiotic susceptibility testing, haemolysis, and DNase tests) and *in vivo* assays (sub-acute oral toxicity tests in male albino rats).
Results: A total of 5 LAB isolates were preliminarily identified from the cabbage. The LAB isolate that showed strong antibacterial activity was confirmed as *Levilactobacillus brevis.* The isolate showed 83.3% survival rate in low pH of 3 and 85.9% in 0.3% bile concentration indicating potential tolerance to gastrointestinal conditions. The cell surface hydrophobicity assay showed 51% and auto-aggregation of 60% which represents the adhesion properties of the isolate. The sub-acute oral toxicity evaluation of the putative probiotic strain in Wister albino rats showed no altered health condition.
Conclusion: The findings in this study suggest that *Levilactobacillus brevis* isolated from Sauerkraut is suitable as probiotics and could be applied in the pharmaceutical and food industry.

Keywords: probiotics; safety assessment; *Levilactobacillus brevis*; sauerkraut; cabbage

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Propriétés antimicrobiennes, innocuité et attributs probiotiques des bactéries lactiques isolées de la choucroute

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Résumé:

Contexte: Selon l'Organisation mondiale de la santé, les probiotiques ont été définis comme des micro-organismes vivants qui, lorsqu'ils sont administrés en quantité appropriée, procurent des avantages pour la santé de l'hôte. Cette étude vise à isoler les bactéries lactiques (LAB) de la choucroute et à déterminer leur potentiel anti-pathogène et leur aptitude en tant que probiotiques.
Méthodologie: Des bactéries lactiques (LAB) ont été isolées à partir de chou fermenté obtenu sur un marché libre à Akure, dans l'État d'Ondo, au Nigeria, en inoculant la suspension de chou préparée sur des plaques triplet de gélose Rogosa (*Lactobacillus* sélectionnant de la gélose) et en incubant dans un bocal anaérobie avec gaspaks à 37°C pendant 72 heures. Les isolats de LAB ont été présumément identifiés phénotypiquement par la

morphologie des colonies, la réaction de coloration de Gram et le test de catalase. L'activité antibactérienne des isolats de LAB a ensuite été réalisée en utilisant des méthodes de superposition d'agar et de diffusion en puits d'agar. L'isolat de LAB le plus efficace a été sélectionné en fonction de sa forte activité antibactérienne, confirmée par le séquençage de l'ARNr 16S et évaluée plus avant pour les activités probiotiques, notamment la résistance aux sels biliaires, la survie à un pH bas, l'hydrophobicité de la surface cellulaire, l'auto-agrégation et la co-agrégation. L'isolat putatif de LAB probiotique a également été évalué pour sa sécurité à l'aide de tests in vitro (tests de sensibilité aux antibiotiques, hémolyse et tests de DNase) et in vivo (tests de toxicité orale subaiguë chez des rats albinos mâles).
Résultats: Un total de 5 isolats de LAB ont été préalablement identifiés à partir du chou. L'isolat LAB qui a montré une forte activité antibactérienne a été confirmé comme étant *Levilactobacillus brevis*. L'isolat a montré un taux de survie de 83,3% à un pH faible de 3 et de 85,9% à une concentration de bile de 0,3% indiquant une tolérance potentielle aux conditions gastro-intestinales. Le test d'hydrophobicité de la surface cellulaire a montré 51% et une auto-agrégation de 60%, ce qui représente les propriétés d'adhérence de l'isolat. L'évaluation de la toxicité orale subaiguë de la souche probiotique putative chez les rats albinos Wister n'a montré aucun état de santé altéré.
Conclusion: Les résultats de cette étude suggèrent que le *Levilactobacillus brevis* isolé de la choucroute convient comme probiotique et pourrait être appliqué dans l'industrie pharmaceutique et alimentaire.

Mots-clés: probiotiques; évaluation de la sécurité; *Levilactobacillus brevis*; choucroute; chou

Introduction:

Microorganisms have historically been an integral part of the human diet, absorbed through naturally fermented foods such as fruits and vegetables and their juices. Lactic acid bacteria (LAB) are common microorganisms that may be found in any environment that is high in carbohydrates, such as plants and other fermented foods (1). The use of preserved and fermented vegetables in human nourishment has been around since ancient times, with sauerkraut being one the most widely consumed vegetable fermented products (2). Sauerkraut, which is a fermented cabbage food with origin in Central Europe, has traditionally been seen as an underutilized natural source of probiotic bacteria that might be used as a starting culture in other fermentation procedures (3).

The health promoting qualities of Sauerkraut have been attributed to its high contents of bioactive components produced as a result of indigenous microbial metabolic activities (4). *Leuconostoc mesenteroides*, *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Pediococcus,* and *Enterococcus* are among the known beneficial bacteria that contribute to sauerkraut formation. Most of the beneficial bacteria with a proven history of safe use are probiotic bacteria.

The human gastrointestinal tract (GIT) and fermented dairy products are the two main sources of probiotic bacteria. The strains derived from other sources, such as plant-based meals, however, provide significant options since they might be more helpful and viable for use in comparable non-dairy-based probiotic products (5). Genetically, plant-derived LAB strains are comparable to milk-derived bacteria and have been shown to exhibit extra advantageous traits such as greater tolerance to stress conditions and the ability to ferment a wider range of carbohydrate sources (6,7).

Probiotics are gaining popularity due to their proven safety and positive benefits on human health (8). The majority of probiotics are members of the LAB group, which includes many species such as *Lactobacillus*, *Leuconostoc, Streptococcus*, and *Enterococcus*. Recent growth in the worldwide market for probiotics has been fuelled by increased consumer demand for healthy diets and well-being, prompting food researchers and manufacturers to produce novel probiotic-containing products. However, there has been growing concern regarding the safety of probiotic bacteria which include the potential to cause diseases such as bacteraemia or endocarditis, production or release of toxic metabolites on the gastrointestinal tract, and the presence of transferable antibiotic resistance genes (9).

Conversely, pieces of evidence that probiotic bacteria are causal agents in cases of the diseases highlighted are limited and unclear, and infection caused by probiotics has never been observed in healthy persons (10). The ability to adhere to human mucosal or epithelial cells and cell lines, exert an antimicrobial effect against pathogenic bacteria or fungi, reduce pathogen adhesion to surfaces, stimulate bile salt hydrolase activity, and increase probiotic viability are among the most crucial characteristics of probiotics (11). This study aimed to isolate possibly probiotic LAB from fermented cabbage (Sauerkraut) and assess the possibility of safe use.

Materials and method:

Preparation of Sauerkraut:

The cabbage used in this study was purchased from Shasha market, Akure, Ondo State, Nigeria. The cabbage was washed in sterile distilled water, and shredded into bits before being placed into a clean glass jar, and 2% salt was added to make 200 ml volume. The shredded cabbage was comple-

tely submerged in distilled water and sealed to ensure anaerobic conditions. The setup was kept at room temperature (ambient temperature of 18-22^{o}C) to ferment for 7 days.

Isolation of and characterization of LAB:

The isolation of probiotic strains from fermented cabbage was carried out according to the method previously described by Jagannath et al., (12). The fermented cabbage was crushed into a slurry in a warring blender. One millimeter was aseptically collected and diluted serially in saline solution. Thereafter, 100µL of each of the diluted suspension (10^{-1} to 10^{-6}) was separately inoculated on triplet plates of Rogosa agar media (De Man Rogosa and Sharpe *Lactobacillus* Selecting Agar, pH 5.4 ± 0.2, Oxoid, CM0627) and spread using a sterile spreader. The inoculated plates were incubated under anaerobic conditions using an anaerobic gas jar containing gaspaks (AnaeroGen™ 3.5L, ThermoScientific, AM0035, UK) at 37^{o}C for 72 hours. The LAB isolates were presumptively identified phenotypically by colony morphology, Gram staining, and catalase test.

Antibacterial & antagonistic activities of LAB

Agar overlay assay:

The indicator bacteria (*Salmonella* Typhi, *Escherichia coli* and *Staphylococcus aureus*) were grown on nutrient agar at 37°C for 24-hour. The inhibitory activity of the selected LAB isolates against the indicator organisms was done using the agar overlay technique described by Hockett and Baltrus (13). The LAB isolates were spot-plated on MRS agar and incubated for 24 hat 37˚C under anaerobic conditions using a gas generating system (AnaeroGen™ 3.5L, Oxoid, AM0035, UK). Cultures of the pathogens were grown on NA agar for 18 hours, and about 3-5 colonies were picked and suspended in sterile saline solution and the turbidity was adjusted to 0.5 McFarland standard equivalent of $1x10^{8}$ CFU/ml. One hundred microliters of the adjusted suspension of indicator strains were seeded in semi-solid Mueller Hinton agar. The MRS agar containing pre-spotted LAB isolate was overlaid with 10 ml soft MH agar containing 100µL seeded indicator organism and incubated aerobically at 37˚C for 24 hours. Clear zones of inhibition of more than 1 mm extending laterally from the border of the LAB colonies after 24 hours incubation was considered positive antagonistic (antibacterial) activity.

Agar-well diffusion test:

Agar–well diffusion assay was carried out to determine the zone of inhibition of the antibacterial agent in the cell-free culture supernatant (CFCS) of LAB isolates against the indicator organisms. Lactobacilli that showed a zone of inhibition in the agar overlay assay were selected and cultured overnight in MRS broth at 37˚C for 18 hr. After incubation, the culture was centrifuged at 10,000 rpm for 10 min at 4˚C and further filtered through a 0.22 µm sterile syringe filter (PES, AXIVA) to obtain a CFSC. Overnight cultures of the indicator bacteria were inoculated in BHI broth and incubated for 3 h at 37˚C. Thereafter, 0.5 McFarland standard suspensions of the culture were prepared.

One hundred microliters of the prepared suspension were mixed with 10ml of melted and cooled semi-solid BHI agar and poured into the respective Petri dishes. The semi-solid BHI agar was allowed to set and wells of 6mm diameter were aseptically made using a sterile cork-borer. Bases of the wells were sealed with 30µl melted semi-solid BHI agar. Wells were filled with 100µl of CFCS and then kept in the refrigerator for 3-4 hours to ensure the diffusion of antimicrobial compounds within the media. The plates were hereafter incubated for 18 hours at 37˚C. The diameters of the zone of inhibition that extends radially from the edge of the wells were taken and recorded. Unspent MRS broth was used as a negative control.

Molecular identification of the efficient LAB

Based on its potential probiotic attributes, the efficient LAB isolate was identified by 16S rRNA sequencing. Extraction (isolation) of DNA and PCR (polymerase chain reaction) amplification was carried out using the universal primers 27F-5'AGA GTT TGA TCC TGG CTC AG-3' and 1492R-5'GGT TAC CTT GTT ACG ACT TT-3'. The reaction was carried out in 25 µL of the reaction mixture, which contained forward and reverse primers (10 pmol), 0.5 µL of DNA template (50–100 ng), dNTP (0.2mM), and polymerase (0.5U). Optimum conditions for the PCR involved an initial denaturation step for 5 min at 95°C followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 55°C, extension for 5 min at 72°C, and final extension for 7 mins at 72°C. The PCR products were confirmed on 1% agarose gel electrophoresis. The PCR products were further sequenced and the obtained sequences were compared using BLAST (basic local alignment search tool).

Evaluation of probiotic attributes of LAB

Cell surface hydrophobicity:

The *in vitro* bacterial cell surface hydrophobicity of LAB isolate-3 (cbak3) was evaluated by measuring the microbial cell adhesion to hydrocarbons (xylene) according to the method described by Rokana et al., (14).

The young cultures in MRS broth were centrifuged at 6,000 rpm for 10 min, washed twice with PBS, and re-suspended in PBS buffer. A cell suspension of about 3ml was blended with 2 ml of xylene and incubated at 37 °C for 3 h without shaking the separation of the aqueous and organic phases. One millimeter of the aqueous phase was removed carefully and the absorbance (A_1) was measured at 600 nm. The cell hydrophobicity assay was measured (in percentage) using the formula; cell surface hydrophobicity % = $[1-A_t/A_0] \times 100$, where A_0 and A_t are the absorbance before and after extraction with organic solvents, respectively.

Acid and bile tolerance assay:

The tolerance of the LAB isolates to both acidic pH and bile salts was carried out using the methodology described previously by Fadare et al., (5) with little modification (pH adjusted to 3.0). Young cultures of the strains were inoculated in 50 ml of MRS broth with an adjusted pH value of 3.0 using 1 M HCl, and 50 ml MRS with 0.3% (w/v) bile salt (Himedia, India), respectively. The inoculum size was adjusted by comparing the turbidity with 0.5 McFarland standards equivalent to ~1.5 x 10^8 CFU/ml. The samples were incubated at 37°C. At time intervals of 0, 1, 2, and 3 h, a 100 µL aliquot was taken to determine the total viable cells by the standard plate count method. The survival rate was determined using the formula; survival rate (%) = Biomass at time (B_t)/Biomass at initial time (B_o) × 100.

Auto-aggregation assay:

The ability of the LAB isolate to auto-aggregate was tested using the method previously described by Zommiti et al., (16). The overnight culture was harvested by centrifugation (at 6000 rpm for 10 min) and washed with PBS twice and re-suspended in PBS buffer. The sample was allowed to stand while incubating anaerobically at 37°C. Afterward, the upper suspension was checked for absorbance at 600 nm at time intervals of 0, 1h, 2h, and 3h. The auto aggregation was measured in percentage using the formula; auto aggregation % =$[1-A_t/A_0] \times 100$, where where A_t represents the absorbance at time t = 3 h and A_0 the absorbance at t = 0.

Co-aggregation assay:

The ability of the LAB isolates and pathogenic indicator organisms (*Escherichia coli* and *Staphylococcus aureus*) to co-aggregate was determined using the method described by del Re et al., (17). The overnight culture was harvested by centrifugation at 6000 rpm for 10 min and washed with PBS twice and re-suspended in PBS buffer. An equal volume of the LAB isolates and pathogenic indicator suspension was prepared with the turbidity equivalent to 0.5 MacFarland standard and allowed to stand for a while. The upper suspension was checked for absorbance at 600 nm at time intervals of 0, 1, 2, and 3 h. Percent co-aggregation was calculated using the equation of Nagaoka et al., (18); co-aggregation % = $[1 - A_t/A_0] \times 100$, where A_t represents the absorbance at time t = 1, 2 or 3 h, and A_0 is the absorbance at t = 0.

Evaluation of the safety of the LAB isolate:

Antimicrobial susceptibility test of LAB:

The AST of the suspected *Lactobacillus* spp. was performed using the Kirby-Bauer disc diffusion technique. The procedure for the experiment was carried out as specified in the Clinical and Laboratory Standard Institute (19) guideline. Mueller–Hinton agar and Brain heart infusion agar (i. e. MHA and BHI in 1:1) were used as previously described (20). The following antibiotic discs were tested; ampicillin (10µg), chloramphenicol (30µg), tetracycline (30µg), gentamicin (10µg), cefuroxime (30µg), erythromycin (15µg), ciprofloxacin (5µg) and vancomycin (30µg).

Suspensions of overnight cultures of the test organisms were prepared with turbidity equivalent to 0.5 McFarland standards in sterile normal saline. The bacterial suspensions were aseptically inoculated on the prepared medium using a sterile swab stick, incubated anaerobically at 37°C for 24 h and then 72 h. The diameter of the zone of inhibition was measured and recorded to the nearest millimeter using a ruler. The susceptibility status was determined using the zone size interpretative chart as provided by CLSI (19).

Haemolysis

The method described by Yadav et al., (21) was used to assess the LAB isolates' haemolytic activity. The isolates tested were streaked onto blood agar plates containing 5% (w/v) human blood and incubated at 37°C for 24 h. The plates were examined for α-, β-, and non-haemolytic activity after incubation.

DNase activity:

The DNase activity of the LAB isolates was determined using the method as previously described by Shuhadha et al., (22). Briefly, the LAB isolates were streaked onto DNase agar medium (Himedia, India) to test for the production of the DNase enzyme. The plates were then incubated at 37°C for 24 h and observed for clear zones showing DNase activity.

***In vivo* sub-acute oral toxicity assay in laboratory animals**

The procedures involving animals in this study were carried out following the guidelines of the Ondo State health research ethics committee. Eight male Wistar rats of the subspecies *Rattus norvegicus* aged 6-7 weeks old, and weighing 68-76 g were obtained from the animal house of Elizade University. The rats were randomly grouped into two (A & B), comprising four rats per group, and allowed to acclimatize for 14 days before beginning the toxicological assay.

The sub-acute oral toxicity assay was a 28-day repeated-dose oral toxicity assay carried out according to the OECD guidelines. The *L. brevis* was revived and prepared for administration using the method previously described by Pradhan et al., (10) with few modifications. Briefly, the bacterial isolates were sub-cultured and finally harvested by centrifugation at 4500 rpm for 15 min after 16 h of incubation at 37^{o}C. The centrifuged culture was thereafter washed thrice in sterile phosphate-buffered saline (PBS). The pellet obtained was then re-suspended in sterile saline and the turbidity was adjusted to 0.5 McFarland standard equivalent to ~1.5 x 10^{8} CFU/ml). Rats in group A were administered oro-gastrically with 500 µL of the bacterial suspension daily for 28 days, while the control group (B) was fed with sterile saline. During the treatment period, daily observations for any changes in the animal's activity were made, and body weight increase was assessed weekly. All rats were sacrificed on the 29th day.

Following the period of toxicity test, blood samples were collected on the 29th day into appropriate vials for haematological analysis. The haematology tests, which include packed cell volume (PCV), haemoglobin concentration (HB), red blood cell count (RBC), erythrocyte sedimentation rate (ESR), and white blood cell (WBC) counts were carried out according to the standard procedure. After the physical evaluation, the rats were sacrificed, the abdominal cavities were opened, the intestines removed and preserved in 10% formalin for histopathological analysis. The tissue sections were prepared on a slide and stained with haematoxylin and eosin. The integrity of the sectioned intestines was examined using a microscope. The intestines of the control group were compared with the treated group.

Statistical analysis

All tests were conducted in triplicates and data were presented as mean and standard deviation, and analysed using the Statistical Package for the Social Sciences (SPSS) version 21.0. To determine significant differences between means, a one-way analysis of variance (ANOVA) was used, with a significant difference of $p < 0.05$.

Results:

In this study, five LAB isolates were recovered from fermented cabbage (Sauerkraut). The pH of the fermented cabbage after 7 days of fermentation was observed to drop from the initial mean value of 6.8±0.23 to a mean value of 3.9±0.15, at temperature of 25^{o}C.

Antibacterial activity of LAB isolates

The five isolates showed antimicrobial activity against the test pathogens in an agar-overlay method, but only the isolates designated as cbak2 and cbak3 showed a zone of inhibition when the cell-free culture supernatant was tested in an agar diffusion method (Table 1). On the other hand, the pH-neutralized cell-free culture supernatants of the two probiotic LAB showed no inhibitory activity against the test pathogens.

16s rRNA sequencing identification

The sequence analysis of cbak3 isolate using the Basic local alignment search tool (BLAST) program detected 97.64% sequence identity with known species (*Levilactobacillus brevis*). The strain has been deposited into the National Center for Biotechnology Information (NCBI) database with accession number OM262042.

Table 1: Antibacterial activity of cell-free supernatants of the 5 LAB isolates against pathogens

Indicator organisms	**Mean zone diameter of inhibition (mm)**				
	Cbak1	**Cbak2**	**Cbak3**	**Cbak4**	**Cbak5**
Salmonella Typhi	0 ± 0.00	1.2 ± 0.21	14 ± 0.02	0.00	0.00
Staphylococcus aureus	0 ± 0.00	3.1 ± 0.04	16 ± 0.32	0.00	0.00
Escherichia coli	0 ± 0.00	0.8 ± 0.32	14 ± 0.05	0.00	0.00

Values are the mean (± SD) of triplicate samples (n=3); cbak= cabbage sample

Table 2: Mean viable count of the lactic acid bacterial isolates

Strain	**Bile (0.3%) tolerance**			**Acid tolerance (pH=3)**		
	0 hour	**3-hour**	**Survival rate (%)**	**0 hour**	**3-hour**	**Survival rate (%)**
L. brevis	8.28 ± 0.04	7.11 ± 0.16	85.9	8.54 ± 0.02	7.11 ± 0.21	83.3

Values are the mean (± SD) of triplicate samples (n=3)

Table 3: Cell surface hydrophobicity, auto-aggregation and co-aggregation of LAB isolate

Bacteria	**CSH (%)**	**Auto-aggregation (%)**	**Co-aggregagtion (%)**		
			***S.* Typhi**	***S. aureus***	***E. coli***
L. brevis	53.3 ±1.73	60.0 ± 2.99	48.3 ±1.73	65.3 ± 2.85	51.7 ±1.73

Values are the mean (± SD) of triplicate assay; CSH = cell surface hydrophobicity

Probiotic attributes of LAB isolate

The LAB cbak3 isolate that showed strong antibacterial activity was further evaluated for its probiotic and safety status. The *L. brevis* isolate showed appreciable levels of tolerance to 0.3% bile salt and acidic pH 2.0 after 3 exposures to both conditions (Table 2). *L. brevis* showed 85.9% and 83.3% survival rates in 0.3% bile salt and low pH of 2.0 respectively.

The *L. brevis* had a median value (33% < 66%) for both the cell surface hydrophobicity and auto-aggregation assays (Table 3). As indicated, *L. brevis* showed a higher co-aggregation rate with *S. aureus* (65.3±2.85%) compared to the percentage values obtained for *E. coli* (51.7±1.73%) and *Salmonella* Typhi (48.3±1.73%).

Safety evaluation results

As shown in Table 4, *L. brevis* showed resistance to ciprofloxacin, cefuroxime, and vancomycin, but sensitive to erythromycin, gentamicin, ampicillin, tetracycline, and chloramphenicol.

Levilactobacillus brevis showed no haemolytic and DNase activities. With respect to the *in vivo* toxicity test, no death in the rats (both the experimental and control group) was reported. No sign of ill- ness was observed as the experimental rats remained active and healthy throughout the experiment. Compared to the control, a significant increase in the average weight (82.2g to 101.96g) of the rats administered with *L. brevis* was observed, showing a weight gain of 19.3% (Table 5).

The values obtained for the WBC and RBC in group A were significantly different compared to the control group. However, the values for each parameter assayed did not exceed the normal range (Table 6). As shown in Table 7, the white blood cell differential counts for the experimental rats were significantly lower ($p < 0.05$) for monocytes and higher for lymphocytes compared to that of the control group. However, the values did not exceed the normal range.

Table 4: Antibiotic-resistant profile of *Levilactobacillus brevis*

Test Bacteria	**Diameter of zone of inhibition (mm)**							
	CIP (5µg)	**E (15µg)**	**CXM (30µg)**	**GEN (10µg)**	**TET (30µg)**	**AMP (10µg)**	**C (30µg)**	**V (30µg)**
L. brevis	R	S	R	S	S	S	S	R

CIP – Ciprofloxacin, E – Erythromycin, CXM – Cefuroxime, GEN – Gentamicin, TET – Tetracycline, AMP – Ampicillin, C – Chloramphenicol. V – Vancomycin; R – Resistant; S – Susceptible; I - Intermediate

Table 5: Effect of putative probiotic bacteria (*L. brevis*) on the weight of Wister albino rats

Group	**Weight gain/week (g)**					***WG (%)**
	0	**1**	**2**	**3**	**4**	
A (*L. brevis*)	82.20±1.05^{a}	86.11±3.19^{a}	92.25±2.05^{c}	97.04±1.16^{d}	101.96±2.18^{d}	19.3
C (Control)	84.30±1.10^{c}	87.49±3.14^{d}	91.66±2.24^{b}	94.22±1.52^{b}	96.68±2.12^{b}	12.8

Values were expressed as mean ± SD of 4 determinations; Values with different superscripts in a row were significantly different ($p < 0.05$); WG = Percentage weight gain after treatment

Table 6: Effect of *L. brevis* on the haematological parameters of Wister albino rats

Group	**Blood Parameter**				
	WBC ($\times10^9$/mm^3)	**PCV (%)**	**HB (%)**	**RBC (/mm^3)**	**PLT ($\times10^3$/mm^3)**
A (*L. brevis*)	9.53±0.11^{d}	42.67±0.58^{a}	14.20±0.20^{a}	39.97±0.19^{a}	11.50±0.17^{a}
B (Control)	7.73±0.07^{b}	46.00±0.00^{b}	15.17±0.33^{b}	35.03±0.17^{b}	11.73±0.24^{a}
*Normal range	4.4 – 14.8	36 – 54	10.4 – 16.5	38 – 66.8	6.38 – 11.8

Values were expressed as mean ± SD of 4 determinations in each group; Values with different superscripts in a column were significantly different ($p < 0.05$); WBC= White blood cell; HB = Haemoglobin; PCV = Park cell volume; RBC = Red blood cell; *Delwatta et al., (23)

Table 7: Effect of *L. brevis* on WBC differential counts of rats

Group	**Parameter**				
	Lymphocytes (%)	**Neutrophils (%)**	**Monocytes (%)**	**Eosinophils (%)**	**Basophils (%)**
A (*L. brevis*)	75.6 ± 0.58^{c}	21.0 ± 1.00^{a}	2.0 ± 0.00^{a}	1.0 ± 0.00^{a}	0.0 ± 0.00^{a}
B (Control)	73.6 ± 0.58^{b}	20.7 ± 0.58^{a}	4.6 ± 0.58^{c}	1.0 ± 1.00^{a}	0.0 ± 0.00^{a}
*Normal range	65 – 85	13 – 36	0 – 5	0 – 6	0 – 1

Values with different superscripts in a row were significantly different ($p < 0.05$). *Delwatta et al., (23)

The histopathology of the intestine of the experimental rats compared with the control group shows no negative pathology architecture. Mucosal epithelia are intact with adequate thickness. The villi are prominent and the integrity is preserved. There was no necrosis, haemorrhage, or kariolysis of the intestinal villi. The intestinal villi projections are well-formed with adequate arterial and venous supply in both the experimental group (a) and the control (b) with adequate projections (Fig. 1a and b).

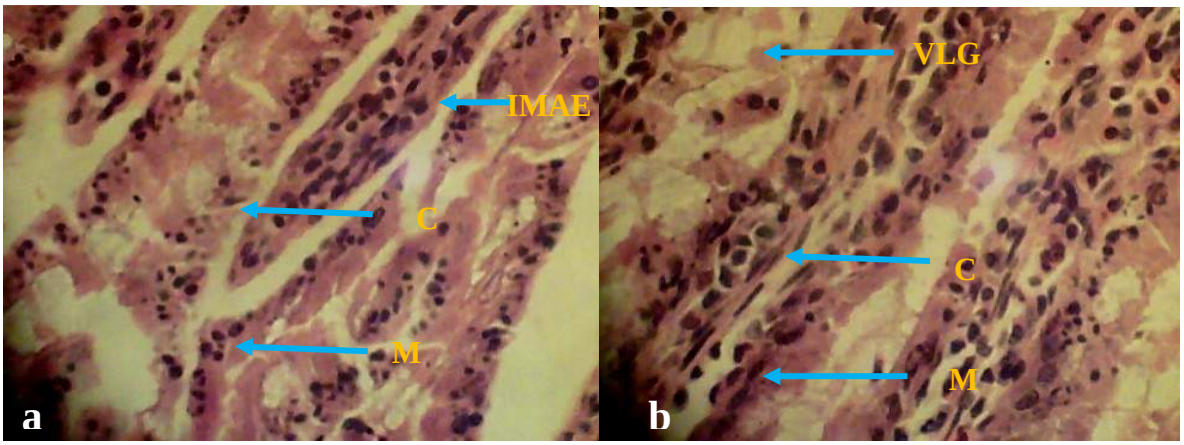

Fig. 1. Photomicrograph of the small intestines of the experimental rats and the control group. **a.** Experimental group administered with 500 µL aliquot of *L. brevis* isolate once daily, **b. C**ontrol group administered with sterile saline solution.
Key: IMAE – Intact mucosal absorptive enterocytes, **VLG** – Villi lining goblets, **C** – Crypt, **M** - Mucosal

Discussion:

Overall, five LAB isolates were successfully recovered from the fermented cabbage, however, only one LAB isolate that showed strong antibacterial activity was selected and identified as *Levilactobacillus brevis*. The putative probiotics in this study were prescreened using antibacterial activity as the primary criterion. As evident in this study, the occurrence of *L. brevis* among other LAB in fermented cabbage (sauerkraut) has been documented (24). *L. brevis* was discovered to have a substantial antagonistic effect on the test foodborne pathogens in this study. In a recent study by Utama et al., (25) *L. brevis* isolated from fermented cabbage was reported to show strong inhibition of *E. coli* and *Salmonella pullorum*. Other studies have also reported strong antimicrobial activity of *L. brevis* isolated from non-vegetable fermented sources against foodborne pathogens (26).

Initially, all five isolates showed high levels of inhibitory activity when tested using the agar-overlay method, but when tested using the agar well diffusion method, *L. brevis* displayed visible and high inhibitory activities against the indicator organisms. This is in tandem with the study conducted by Polak-Berecka et al., (27), where the agar spot test method showed the largest and most distinct inhibitory zones compared to the agar diffusion technique. However, the result obtained in this study using the two methods (agar overlay and agar diffusion) was at variance with the report published by Halder et al., (28), in which the values of results obtained using both methods were in close range. The variation observed in the antibacterial activities between the two methods and as reported by different authors have been attributed to cell density and growth kinetics of the LAB used in the agar overlay methods (29). On the other hand, the volume of cell-free supernatant culture used for the diffusion test has also been considered a potential factor (28). According to Polak-Berecka et al., (27), when live *Lacticaseibacillus rhamnosus* cells were employed instead of cell-free supernatant, the strongest antibacterial activity was seen.

The presence of antimicrobial compounds such as organic acids (lactic, citric, acetic, fumaric, and malic acid), hydrogen peroxide, CO_2, diacetyl, ethanol, reuterin, acetaldehyde, acetoin, ammonia, bacteriocins, bacteriocin-like inhibitory substances (BLIS), and other important metabolites have been attributed to the antibacterial activity of *Lactobacillus* spp (30,31). To guarantee colonization, survival, and metabolic activity within the gut, probiotic isolates must be able to survive bile salts and low pH, otherwise, they will not be able to benefit the host's health and well-being. Tolerance to acidic environments and bile salts have been regarded as two characteristics for a prospective probiotic strain to survive the GIT (32). The pH value of 3.0 and concentration of 0.3% bile salt have been considered standard for such investigation of probiotic strains (33). In the current study, *L. brevis* showed a higher survival rate within the 80% range in both 0.3% bile and acid (pH 3.0) tolerance tests. *Lactobacillus* has been reported to survive and proliferate lavishly in MRS broth enriched with 0.3% bile salt (34), which is similar to our observation. According to Shokryazdan et al., (35), *Lactobacillus* isolates were acid-tolerant for 3 h at pH 3.0. Ehrmann et al., (36) discovered that the acid tolerance level differed across *Lactobacillus* isolates tested under identical conditions, suggesting that tolerance capability may be strain-specific. It is crucial to note, however, that *in vitro* studies involving pH and bile salt tolerance

cannot predict patterns of behavior in the human body. The stomach pH which is usually within the range of 1.2 – 2.0 has been reported to fluctuate depending on the meal consumed. The stomach pH has been shown to rise dramatically close to 5.0 after ingesting yogurt and fermented milk (37).

Cell surface hydrophobicity is another attribute that has been associated with probiotic strain adhesion capacity (5). A correlation between surface hydrophobicity and bacteria capacity to adhere to the intestinal mucosa has been reported (5). In the current study, the hydrophobic character of the surfaces of *L. brevis* was evaluated based on its adhesion to the hydrocarbon phase in a xylene solution. *Lactobacillus brevis* showed a higher hydrophobicity percentage (51%), similar to the result obtained by Kariyawasam et al., (38) who used xylene in their study. Meanwhile, the hydrophobicity of *L. brevis* G1 and *L. brevis* KU15006 in another study ranged from 47% to 48% (39).

It has been demonstrated that pathogens frequently have a high affinity for proteins including collagen, fibrinogen, and mucus because they provide them access to host tissues (40). Auto-aggregation refers to a bacterial strain capacity to interact with itself in a generic manner, which is thought to be a precondition for pathogen colonization and infection of the gastrointestinal tract via adhesive ability. The percentage value of auto-aggregation obtained in the current study was high for *L. brevis*, although the isolate showed values found within the acceptable range (17). Strains of *Lactobacillus* in general have been shown to have a low to moderate auto-aggregation capability (41). As per the co-aggregation test, *L. brevis* showed a higher aggregation percentage with *S. aureus* compared to *E. coli* and *S.* Typhi. A similar pattern of activity has been documented, with the highest levels of co-aggregation reported between the Lactobacillaceae and *E. faecalis* strains and the lowest levels with *S*. Typhimurium and *E. coli* (42). Generally, most LAB which are Gram-positive have been shown to exert low co-aggregating capabilities against Gram-negative bacteria (5,43). It is not clear why this is so, but the structural differences between the cell wall of Gram-negative and Gram-positive bacteria may be a determining factor. Variations by species in the physiology and surface features of cells such as polysaccharide content and adhesin expression have been documented (44). Probiotic bacteria have been shown in several studies to inhibit harmful bacteria from adhering to the intestinal mucosa by creating a physical barrier via auto-aggregation or co-aggregation with the pathogens (45,46).

The lack of acquired antibiotic resistance genes in bacterial strains authorized for use in human and animal feed is a requirement of the European Food Safety Authority (47). The fundamental worry with antibiotic resistance features is their tendency to spread to other potentially harmful bacteria, possibly causing difficulties and reducing the efficacy of antibiotic therapy. In the current study, *L. brevis* was sensitive to clinically relevant antibiotics including ampicillin, tetracycline, chloramphenicol, gentamicin, and erythromycin which are following the EFSA guidelines (47). Our isolate in line with previous studies showed resistance to ciprofloxacin and vancomycin (48,49). Vancomycin resistance in *L. brevis* isolates has been demonstrated to be typically high, and the resistance is thought to be an inherent trait of the strains (48,50). Additionally, the fact that our strains are of plant origin suggests that they have not come into contact with antibiotics, which might account for the resistance as an inherent trait, in contrast to isolates from humans and animals.

Our study showed that *L. brevis* has non-hemolytic and non-DNAse activities, which comply with the suggested safety features in probiotic selection (51). The pathogenic nature of some groups of bacteria has been attributed to DNase and haemolytic activities (52), and many studies have demonstrated the non-haemolytic and non-DNAse activities of *Lactobacillus* spp. isolated from different sources including humans, animals, and plants (53,54). With regards to the *in vivo* toxicity test, the outcomes of repeated oral administration of *L. brevis* to a group of four albino rats further support the non-pathogenic attribute of the test isolate. To ascertain the safety of a bacterial strain, oral toxicity studies have been considered a standard (9,55). Based on the results of a repeated-dose sub-acute oral toxicity study by Shokryazdan et al., (35), none of the tested *Lactobacillus* strains caused any sign of toxicity when fed to rats at a concentration as high as 1×10^{10} CFU/kg BW/day. The significant weight gain of the albino rat test group observed in this study may be attributable to increase nutrient absorption which may have been aided by the enzyme activity of *L. brevis*. This finding appears to be consistent with a recent work by Oladejo et al., (56), in which hydrolyzed African yam beans with partially purified protease produced by *L. brevis* increased vitamin bioavailability, which accounted for the albino rats' bodyweight gain. However, weight loss caused by some strains of *L. brevis* and other species of *Lacto bacillus* has been reported (57,58), which suggest species and strain specificity (59).

Haematological analysis is vital for assessing toxicological consequences such as inflammation and infection caused by induced treatments (60). In this study, the levels of haemoglobin, packed cell volume, platelet and red blood cells were within the normal range, which could suggest a reduced risk of anaemia. Moreover, WBC count, an indicator for blood infection, was equally within the normal range despite being higher than in the control group. In the treated group, no such abnormality in the differential counts of lymphocytes, neutrophils, monocytes, eosinophils, and basophils was observed. There is a sparse report in the literature showing any abnormality in the blood profiles of healthy albino rats administered with *Lactobacillus* species.

The integrity of the gut was further verified by histology of the intestine. The microscopic observations of the stained tissue of the intestine did not reveal any signs of inflammation, degeneration, or necrosis of the intestinal mucosa, and there was no remarkable difference in the arrangement of epithelial cells between the test and control groups. Although, the histology of the intestine showed that the formation of intestinal finger-like villi differs in maturity, no structural changes in the crypt depth or distortion in the villus structure were observed. Probiotics have been intensively studied for their involvement in maintaining the intestinal barrier, which is thought to be the first line of defense against pathogens entering the digestive system (61,62). Reinforcement of gut barrier function can lead to decreased intestinal inflammation, and several investigations have shown that probiotic strains can restrict inflammatory responses by strengthening gut barrier permeability (63,64).

Conclusion:

Based on the findings of this study, it can be stated that the *L. brevis* isolated from Sauerkraut is potentially safe and useful due to its appealing probiotic features and promising antibacterial activity. This lends credence to the idea that as a probiotic strain, it might be useful in a variety of applications, particularly in the food industry. However, whole strain genomic profiling as well as clinical trials are further required to affirm the suitability of the putative probiotic isolate before it can be applied in human or animal nutrition.

Contribution of authors:

OSF, CHA, AOM and TKB conceptualized and designed the study. Material preparation, data collection, and analysis were carried out by OSF, CHA and AOM. The first and second draft of the manuscript were written by CHA and OSF respectively. All authors discussed the results and contributed to the final manuscript draft for submission.

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Original Article **Open Access**

Bacterial contaminants of Date palm fruits *(Phoenix dactylifera)* sold in Kaduna, Nigeria, and their susceptibility to antibiotics

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Abstract:

Background: Antibiotic resistance is a major challenge of antimicrobial therapy of infections today. Food-borne bacteria can serve as reservoir for transmission of antibiotic resistant strain. This study was aimed at determining the bacterial contaminants on dates palm fruits sold in Kaduna metropolis and to determine the antibiotic resistance pattern of the bacteria isolated.
Methodology: A total of 60 samples of Date palm fruits (*Phoenix dactylifera* L.) were collected randomly from five major markets in Kaduna metropolis. Total bacterial and total coliform counts were determined using pour plate method. Gram, staining, microscopy and biochemical tests were carried out to isolate the bacterial contaminants. Antibiotic susceptibility test for commonly prescribed antibiotics was also carried out through agar diffusion method and the percentage antibiotic resistance determined. Data were analysed using descriptive statistics on Microsoft Excel.
Results: The results showed that the mean total bacterial count range was 4×10^5-2.7×10^6. The mean coliform count range was 8×10^3-1.2×10^6. The following bacteria were isolated; *Staphylococcus aureus* (35.7%), *Streptococcus* spp (21.4%), *Escherichia coli* (12.5%), *Bacillus subtilis* (8.9%), *Enterobacter* spp (8.9%), *Proteus mirabilis* (7.1%) and *Salmonella* Typhi (5.4%). Gentamicin (43.2%) and cotrimoxazole (47.4%) were the most active against Gram-positive and Gram-negative isolates respectively. The bacterial isolates showed high level of antibiotic resistance with 57.1% being multidrug resistant, 10.7% extensively resistant and 5.4% were resistant to all the antibiotics tested.
Conclusion: The high level of contamination and antibiotic resistance observed in this study is alarming and of public health concern, as Date fruit is highly consumed in this part of the country. Thorough washing of Date fruits with clean water before eating is recommended.

Keywords: Date palm fruits, Resistance, Antibiotics, Bacteria

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Contaminants bactériens des fruits du palmier dattier (*Phoenix dactylifera*) vendus à Kaduna, Nigeria, et leur sensibilité aux antibiotiques

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Résumé:

Contexte: La résistance aux antibiotiques est aujourd'hui un défi majeur de la thérapie antimicrobienne des infections. Les bactéries d'origine alimentaire peuvent servir de réservoir pour la transmission de souches résistantes aux antibiotiques. Cette étude visait à déterminer les contaminants bactériens sur les fruits du palmier dattier vendus dans la métropole de Kaduna et à déterminer le schéma de résistance aux antibiotiques des bactéries isolées.
Méthodologie: Au total, 60 échantillons de fruits de palmier dattier (*Phoenix dactylifera* L.) ont été prélevés au hasard dans cinq grands marchés de la métropole de Kaduna. Le nombre total de bactéries et de coliformes totaux a été déterminé à l'aide de la méthode de la plaque de coulée. Des tests Gram, coloration, microscopie et biochimiques ont été effectués pour isoler les contaminants bactériens. Un test de sensibilité aux antibiotiques pour les antibiotiques couramment prescrits a également été effectué par la méthode de diffusion sur gélose et le pourcentage de résistance aux antibiotiques a été déterminé. Les données ont été analysées à l'aide de statistiques descriptives sur Microsoft Excel.
Résultats: Les résultats ont montré que la plage de numération bactérienne totale moyenne était de 4×10^5 à $2,7\times10^6$. La plage de numération moyenne des coliformes était de 8×10^3-$1,2\times10^6$. Les bactéries suivantes ont été isolées; *Staphylococcus aureus* (35,7%), *Streptococcus* spp (21,4%), *Escherichia coli* (12,5%), *Bacillus subtilis* (8,9%), *Enterobacter* spp (8,9%), *Proteus mirabilis* (7,1%) et *Salmonella* Typhi (5,4%). La gentamicine (43,2%) et le cotrimoxazole (47,4%) étaient les plus actifs contre les isolats Gram-positifs et Gram-négatifs, respectivement. Les isolats bactériens ont montré un haut niveau de résistance aux antibiotiques avec 57,1% de multirésistance, 10,7% de résistance extensive et 5,4% de résistance à tous les antibiotiques testés.
Conclusion: Le niveau élevé de contamination et de résistance aux antibiotiques observé dans cette étude est alarmant et préoccupant pour la santé publique, car les dattes sont très consommées dans cette partie du pays. Il est recommandé de laver soigneusement les dattes à l'eau claire avant de les manger.

Mots clés: Fruits du palmier dattier, Résistance, Antibiotiques, Bactéries

Introduction:

Date fruits are one-seeded fruits of date palm tree (*Phoenix dactylifera*), which belong to the family of Palmae (Arecaceae) plant. The common English names include dried dates and date palm. Date fruits are usually oblong, with varying sizes, shapes, colors, consistencies and quality. Date fruit is regarded to as a pivotal crop that is extensively farmed in the Middle East and Africa (1), and these regions are responsible for the exportation of date products worldwide (2). Date fruits are one of the most common fruits consumed by Nigerians. It is known as *Dabino* or *Dabinu* in Hausa language, and is mostly grown and cultivated in the northern part of the country.

Date fruits can be consumed fresh after harvest or can be semi-dried or totally dried for consumption. It is an important component of the diet in most of the hot arid and semi-arid regions of the world, and is known to contain carbohydrates, mostly in form of fructose and glucose (total sugars content is 35-88%), fats (0.2-0.4%), proteins (2.3-5.6%), fibers (6.4-11.5%), and minerals and vitamins (3,4). Other studies have shown that Date fruits contain many bioactive compounds such as anthocyanins, carotenoids, phenolics, sterols, procyanidins and flavonoids, which are thought to have beneficial effects on human health (5-8). They are also rich in polyphenols and functional dietary fiber that help to maintain the functions of the digestive tracts (9,10). The antimutagenic, antioxidant, anticarcinogenic and anti-inflammatory bioactivities of Date fruits have been attributed to the contribution of polyphenols (11).

Microbial contaminants have been isolated from Date fruits, including yeasts, molds, lactic acid bacteria and some potential pathogens such as *Staphylococcus aureus, Escherichia coli*, and *Aspergillus flavus/parasiticus* (12, 13). Bacteria growth can be facilitated by high moisture content of the Date flesh, but on the other hand, growth of molds become prominent when the Dates are dried and stored (14). The increasing rate of bacteria developing resistance to antibiotics globally is alarming, and antibiotic resistant bacterial strains have been isolated from ready-to-eat fruits and vegetables (15). This study therefore aimed to assess the bacteria contaminants in Dates fruits sold in Kaduna metropolis and to determine the antibiotic susceptibility of the isolates.

Materials and method:

Study setting and Date fruit samples collection:

A total of 60 samples of Dates fruits (*Phoenix dactylifera* L.) each wrapped in polythene bags (as was being sold) were purchased randomly from five major locations in Kaduna metropolis, Kaduna State, Nigeria. The locations were Gonin gora, Central market, Sabo tasha, Ungwa Rimi and Kawo markets.

Preparation of Date palm fruits for analysis:

The samples of the Date fruits were cleaned and pitted. About 5g of the fruits were aseptically weighed into sterile stomacher bags and 45ml normal saline was added. Samples

were homogenized for 15 minutes and aliquots were used for microbiological analysis. One milli-liter of the aliquot from each sample was added into a tube containing 9ml of sterile normal saline, thoroughly shaken, and serially diluted up to 10^{-5}.

Determination of bacteria and coliform counts:

A 0.1ml each of the serially diluted sample (10^{-3} and 10^{-4}) were spread over the surface of the nutrient agar plates (for viable bacteria count), and on MacConkey agar plates (for coliform count), with the aid of sterile glass spreader. This was done in duplicates for each dilution. The plates were incubated at 37^0C for 24 hours, following which viable bacteria and coliform colonies were counted to determine the total bacterial and coliform counts, expressed as colony forming unit per millimeter (CFU/ml). Pure colonies from the total bacteria counts were isolated by sub-culturing on fresh nutrient agar plates. The pure cultures were preserved on nutrient agar slants for further studies.

Identification of bacterial isolates:

The culture plates were examined for colony morphology, and bacteria identified following Gram staining reaction, conventional biochemical tests and growth on selective media. The biochemical identification tests performed included catalase, coagulase, methyl red, Voges Proskauer, indole, triple sugar iron, oxidase and citrate utilization tests (16).

Antibiotic susceptibility test:

The agar disc diffusion method was used for antibiotic susceptibility test according to the Clinical and Laboratory Standards Institute (17) guideline. A sub-culture of the bacterial isolate from nutrient agar slant was prepared on nutrient agar plate and incubated aerobically overnight. Colonies from the plate were emulsified aseptically in sterile saline to produce inoculum equivalent to 0.5 McFarland turbidity standard (~1.5×10^8 CFU/ml). A sterile swab was dipped into the standardized inoculum and squeezed gently against the inside of the tube to remove excess fluids. The swab was streaked on sterile Mueller Hinton agar plate and allowed to dry for 5 minutes. Antibiotic discs were then placed on the surface of the agar using sterile forceps, and the plates were inverted carefully and incubated for 24 hours at 37° C. The diameter of zone of inhibition for each antibiotic was measured with a metric ruler and interpreted as sensitive, intermediate or resistant according to the interpretative chart of CLSI (17). The following antibiotic discs (Bio-Rad®, Ca, USA) were used; tetracycline (30µg), gentamicin (10µg), cefoxitin (30 µg), erythromycin (15µg), ceftriaxone (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), amoxicillin-clavulanate (30µg) and trimethoprim/sulfamethoxazole (1.25/23.75µg).

Determination of multiple antibiotic resistance:

The bacterial isolates resistant to at least one agent in three or more antimicrobial categories were classified as multidrug resistant (MDR) while extensively drug resistant (XDR) isolates were defined as those that were non-susceptible to all the antibiotic categories used except one or two. Pandrug resistance (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories used (18).

Statistical analysis:

The data was analysed using descriptive analysis through Microsoft excel.

Results:

The total bacteria viable count from the samples of Date fruits from different sites in Kaduna metropolis varies from $4x10^5$ to $2.7x10^6$ CFU/ml while coliform count varies from 8×10^3 to 1.2×10^6 CFU/ml (Table 1). Samples collected from Ungwan rimi had the highest total bacteria count (2.7×10^6 CFU/ml) and coliform counts ($1.2x10^6$) while Gonin gora had the least bacteria count (4×10^5 CFU/ml).

A total number of 56 bacterial isolates; 19 (33.9%) Gram-positives and 37 (66.1%) Gram-negatives, were recovered on cultures, with *S. aureus* 20 (35.7%) being the most frequent bacteria isolates. Other isolated bacteria were *Streptococcus* spp*, E. coli, Bacillus subtilis, Proteus mirabilis, Enterobacter* spp and *Salmonella* Typhi. Sample collected from Ungwan Rimi market had the highest frequency of bacteria isolates (Table 2).

Table 1: Total viable bacteria and coliform counts of Date fruits from selected markets, Kaduna, Nigeria

S/N	Market sample location	Total viable bacteria count (CFU/ml)	Total coliform count (CFU/ml)
1	Ungwa Rimi (U)	2.7×10^6	1.2×10^6
2	Gonin gora (G)	4×10^5	5×10^4
3	Central (C)	5×10^5	6×10^4
4	Kawo (K)	7×10^5	1×10^5
5	Sabon tasha (S)	2.3×10^6	8×10^3

CFU/ml = Colony forming unit per milliliter

Table 2: Frequency of bacterial isolates of Date fruits from different market sample sites in Kaduna, Nigeria

Bacteria isolates	Central	Ungwa Rimi	Sabo tasha	Gonin gora	Kawo	Total (%)
Proteus mirabilis	1	0	3	0	0	4 (7.1)
Staphylococcus aureus	5	3	3	5	4	20 (35.7)
Streptococcus spp	2	5	1	2	2	12 (21.4)
Bacillus subtilis	1	1	1	1	1	5 (8.9)
Escherichia coli	1	1	2	1	2	7 (12.5)
Enterobacter spp	1	1	1	1	1	5 (8.9)
Salmonella Typhi	0	1	0	1	1	3 (5.4)
Total	**11**	**12**	**11**	**11**	**11**	**56 (100)**

Table 3: Antibiotic susceptibility of Gram positive and Gram-negative bacterial isolates of Date fruits in Kaduna, Nigeria

Antibiotic	Gram positive isolates (n = 37)			Gram negative isolates (n = 19)		
	S	I	R	S	I	R
Tetracycline (30µg)	3 (8.1)	10 (27.0)	24 (64.8)	7 (36.8)	2 (10.5)	10 (52.6)
Ceftriaxone (30µg)	14 (37.8)	9 (24.3)	14 (37.8)	9 (47.4)	4 (21.1)	6 (31.6)
Ciprofloxacin (5µg)	8 (21.6)	8 (21.6)	21 (56.7)	4 (21.1)	12 (63.2)	3 (15.8)
Erythromycin (15µg)	15 (40.5)	11 (29.7)	11 (29.7)	5 (26.3)	3 (15.8)	11 (57.9)
Chloramphenicol (30µg)	11 (29.7)	13 (35.1)	13 (35.1)	7 (36.8)	4 (21.1)	8 (42.1)
Amoxicillin-Clavulanate (30µg)	10 (27.0)	4 (11.8)	23 (62.1)	4 (21.1)	3 (15.8)	12 (63.2)
Gentamicin (10µg)	16 (43.2)	2 (5.4)	19 (51.4)	8 (42.1)	3 (15.8)	8 (42.1)
Co-trimoxazole (1.25/23.75µg)	15 (40.5)	7 (18.9)	15 (40.5)	9 (47.4)	7 (36.8)	3 (15.8)
Cefoxitin (30µg)	11 (29.7)	0	26 (70.3)			

S = Sensitive; I = Intermediate; R = Resistance

Comparing the antibiotic susceptibility of the Gram-positive and Gram-negative isolates, highest susceptibility was observed to gentamicin (43.2%) and cotrimoxazole (47.4%) by Gram-positive and Gram-negative isolates respectively (Table 3), although *E. coli* was highly susceptible to gentamicin (71.4%) (Table 4). Out of the 20 *S. aureus* isolates, 12 (60%) were phenotypically methicillin-resistant *S. aureus* (MRSA). The result of the antibiotic susceptibility of the MRSA isolates showed that 1 (8.3%) was resistant to all the antibiotics tested (pan-drug resistant). The MRSA isolates were highly resistant to all the beta lactam antibiotics tested

(Table 5). The resistant pattern of the Gram-negative isolates showed that 11 (57.9%) were multidrug resistant, 3 (15.8%) were extensively resistant while 1 (5.3%) was resistant to all the antibiotics tested.

Discussion:

Bacterial contamination of fruits and vegetables is a potential source of foodborne infection which is of public health concern. In this study all the Date fruits sampled were highly contaminated. The results of the total bacteria viable count show the level of contamination in the sampled fruits to be higher than the allowed limit of not greater than 10^4 CFU/g (19). The observed bacterial contamination may be from handling, packaging and the environment. The vendors usually use bare hands to pack the fruits into polythene bags before selling. In some places, the fruits are measured directly for sale using milk tins, all of which can contribute to the high total bacteria viable count.

The total bacteria viable count reported in our study agrees with reports of previous studies. Raimi (4) reported similar bacterial load of $4x10^5$ to $2x10^6$ CFU/ml from Date fruits purchased in Owode market Offa, Kwara State, Nigeria, but lower bacterial load was reported by Umar et al., (20) and Aleid et al., (3) from Date fruits sold in Katsina metropolis Nigeria and in Saudi respectively. High coliform count may be an indication of faecal contamination. The values are higher than 10^2 (CFU/g) coliform limit specified by the Public Health Laboratory Services of Saudi Arabia (19,21). Some of the fruits for sale are kept openly in bowls and wheelbarrows in the open market where they are exposed to possible contamination by houseflies. Housefly can, through its vomits or excrements, transmit *E. coli* that causes diarrhoea or other pathogens that cause shigellosis, typhoid fever and cholera (22). Consuming such contaminated fruits constitute a potential health hazard to the society.

In comparison with other studies on microbiological assessment of Date fruits, lower coliform counts were reported (20,23) and the absence of coliform bacteria and *E. coli* was observed in fresh Dates samples in Dhaka city (24). The highest bacteria and coliform count observed from the samples from Ungwa Rimi market might be an indication of the poor state of hygiene of the vendors in that market compared with other markets.

Table 4: Antibiotic susceptibility of Gram-negative bacterial isolates of Date fruits in Kaduna, Nigeria

Antibiotics	***Proteus mirabilis* (n=4)**			***Escherichia coli* (n=7)**			***Enterobacter* spp (n=5)**		
	% S	**% I**	**% R**	**% S**	**% I**	**% R**	**% S**	**% I**	**% R**
Tetracycline (30µg)	50	0	50	28.6	14.3	57.1	60	20	20
Ceftriaxone (30µg)	50	0	50	57.1	0	42.9	20	40	40
Ciprofloxacin (5µg)	25	25	50	14.3	85.7	0	0	80	20
Chloramphenicol (30µg)	25	25	50	28.6	14.3	57.1	80	0	20
Amoxicillin-Clavulanate (30µg)	25	25	50	28.6	0	71.4	20	40	40
Gentamicin (30µg)	25	0	75	71.4	0	28.6	20	20	60
Co-trimoxazole (1.25/23.75µg)	50	25	25	57.1	42.9	0	60	20	20

S = Sensitive; I = Intermediate; R = Resistance

Table 5: Antibiotic resistance of MRSA isolates from Date fruits in Kaduna, Nigeria

Antibiotics	**Resistant n (%)**	**Sensitive n (%)**
Tetracycline (30µg)	11 (91.7)	1 (8.3)
Ceftriaxone (30µg)	8 (66.7)	4 (33.3)
Ciprofloxacin (5µg)	11 (91.7)	1 (8.3)
Erythromycin (15µg)	9 (75.0)	3 (25.0)
Chloramphenicol (10µg)	8 (66.7)	4 (33.3)
Amoxicillin-Clavulanate (30µg)	11 (91.7)	1 (8.3)
Gentamicin (30µg)	6 (50.0)	6 (50.0)
Cotrimoxazole (1.25/23.75µg)	7 (58.3)	5 (41.7)

The pathogenic organisms isolated from the Date fruits in this study included *S. aureus, E. coli, Bacillus subtilis, Streptococcus* spp*, Enterobacter* spp*, S.* Typhi and *P. mirabilis*. *S. aureus* was the most frequent isolate, which is usually carried transiently in the anterior nares of about 30% healthy individuals and about 20% individuals have it on their skin. Through direct tissue invasion, *S. aureus* can cause skin infections, pneumonia, endocarditis, osteomyelitis and infectious arthritis. In a related study (4), similar microorganisms were isolated from Date fruits purchased from Owode market in Offa, Nigeria while *S. aureus, Klebsiella* and *Bacillus* spp were isolated from Date fruits in Ado Ekiti and Akure, Nigeria (25). Each of these isolated organisms are potentially hazardous to human health. Although *E. coli* may be a harmless commensal flora of the gastrointestinal tract, it can cause relatively mild diarrhoea but some *E. coli* pathotypes can cause severe stomach cramps, bloody diarrhoea and vomiting, and extra-intestinal infections such as urinary tract infection, blood stream infections and neonatal meningitis. *Salmonella* Typhi is the causative organism of typhoid fever, which is usually acquired from contaminated food and water (26). Bacteremia, endocarditis, pneumonia and septicemia have also been attributed to *B. subtilis.*

The samples of the Date fruits used in this study were obtained from open market, implying the possibility of cross-transmission of multi-drug resistant bacterial pathogens, with gentamicin and co-trimoxazole being the only tested antibiotics, demonstrating some *in vitro* anti-microbial activity. The issue of antimicrobial resistance has become a great concern globally. Self-medication, purchase of antibiotics over the counter without prescription, proliferation of un-registered patent medicine stores and unrestricted sales of drugs including antibiotics in the open market are all possible factors responsible for increased rate of antimicrobial resistance.

Conclusion:

The Date palm fruits sampled in this study were all contaminated by bacteria pathogens above the official level considered fit for human consumption. High level of antibiotic resistance was also observed among the bacterial isolates, with gentamicin and co-trimoxazole being the only antibiotics demonstrating *in vitro* anti-bacterial actions against the pathogens. It is recommended that there should be increased awareness among the fruit vendors on good personal hygiene, the dangers of consuming contaminated fruits among the general public. Also, increased advocacies about the implications of antibacterial resistance and the methods of prevention in the society are highly recommended.

Contributions of authors:

AFO conceived the idea, designed the study, and wrote the initial manuscript; DSY was involved in sample collection, and MTD was involved in data analysis. All the authors approved the final manuscript.

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Conflict of interest:

Authors declared no conflict of interest.

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Original Article **Open Access**

Association of intestinal helminthic infection and nutritional status of primary school children in Gombe State, Nigeria

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Abstract:

Background: Intestinal helminthic infections are among the commonest infections worldwide. It often affects the poorest communities and has similar geographic distribution with malnutrition. Intestinal helminthic infection contributes to undernutrition through subtle reduction in digestion and absorption of food, chronic inflammation and loss of nutrients. The objective of this study is to determine the prevalence of intestinal helminthic infection and its relationship with nutritional status of primary school children in Gombe, Gombe State, Nigeria.
Methodology: This was a cross sectional study of 350 pupils selected through multistage random sampling technique from 24 primary schools in Gombe, Gombe State, Nigeria from July 2018 to January 2019. Demographic information including age, gender, height, and weight were collected from each participant with a designed collection form. The data were analysed using SPSS version 24.0, and presented as frequency distribution and mean ± SD. The Chi-square test (with Odds ratio and 95% confidence interval) was used to test for association between prevalence of helminthiasis and factors such as gender, age group and school type. A *p* value of less than 0.05 was considered statistically significant at 95% confidence interval.
Results: The prevalence of intestinal helminthic infection was 23.7% (83/350). Eighty (96.4%) of the 83 infected pupils were in public schools while only 3 (3.6%) were in private schools ($p<0.001$). The prevalence of helminthiasis was significantly higher in underweight pupils (34%, OR=2.113, $p=0.0065$)) and significantly lower (5.4%, OR=0.1637, $p=0.0037$) in overweight pupils while the prevalence was not significantly associated with normal weight ($p=0.5482$) or obesity ($p=1.000$).
Conclusions: Intestinal helminthic infection is a public health problem in children with adverse significant relationship with nutritional status. Provision of toilet facilities in schools and periodic de-worming of pupils aimed at reducing loss of nutrients from intestinal helminthiasis are recommended.

Keywords: Intestinal helminths, school, pupils, nutritional status.

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Association de l'infection helminthique intestinale et de l'état nutritionnel des enfants de l'école primaire dans l'État de Gombe, au Nigeria

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Résumé:

Contexte: Les infections intestinales helminthiques sont parmi les infections les plus courantes dans le monde. Elle affecte souvent les communautés les plus pauvres et a une répartition géographique similaire à la malnutrition. L'infection helminthique intestinale contribue à la dénutrition par une réduction subtile de la digestion et de l'absorption des aliments, une inflammation chronique et une perte de nutriments. L'objectif de cette étude est de déterminer la prévalence de l'infection helminthique intestinale et sa relation avec l'état nutritionnel des enfants de l'école primaire à Gombe, dans l'État de Gombe, au Nigeria.

Méthodologie: Il s'agissait d'une étude transversale de 350 élèves sélectionnés par une technique d'échantillonnage aléatoire à plusieurs étapes dans 24 écoles primaires de Gombe, dans l'État de Gombe, au Nigéria, de juillet 2018 à janvier 2019. Des informations démographiques, notamment l'âge, le sexe, la taille et le poids, ont été recueillies auprès de chaque participant avec un formulaire de collecte conçu. Les données ont été analysées à l'aide de SPSS version 24.0, et présentées sous forme de distribution de fréquence et de moyenne ± ET. Le test du chi carré (avec rapport de cotes et intervalle de confiance à 95%) a été utilisé pour tester l'association entre la prévalence des helminthiases et des facteurs tels que le sexe, le groupe d'âge et le type d'école. Une valeur de p<0,05 a été considérée comme statistiquement significative à un intervalle de confiance de 95%.
Résultats: La prévalence des helminthiases intestinales était de 23,7% (83/350). Quatre-vingts (96,4%) des 83 élèves infectés étaient dans des écoles publiques alors que seulement 3 (3,6%) étaient dans des écoles privées (p<0,001). La prévalence des helminthiases était significativement plus élevée chez les élèves en insuffisance pondérale (34%, OR=2,113, p=0,0065)) et significativement plus faible (5,4%, OR=0,1637, p=0,0037) chez les élèves en surpoids, alors que la prévalence n'était pas significativement associée à un poids normal poids (p=0,5482) ou obésité (p=1,000).
Conclusion: L'infection intestinale helminthique est un problème de santé publique chez l'enfant ayant une relation défavorable significative avec l'état nutritionnel. La mise à disposition de toilettes dans les écoles et le déparasitage périodique des élèves visant à réduire la perte de nutriments due aux helminthiases intestinales sont recommandés.

Mots-clés: helminthes intestinaux, école, élèves, état nutritionnel.

Introduction:

Intestinal helminthiases are among the most common infections worldwide. It affects the poorest and most deprived communities (1, 2). The World Health Organization (WHO) estimates that over 1.5 billion people are infected with helminths worldwide (1). Over 270 million pre-school-age children and over 600 million school-aged children live in areas where these helminths are intensively transmitted. The public health burden and prevalence of intestinal helminths in Nigeria are increasing, with 45% of school-age children infected annually (3,5,6). Globally, about 100 million children experience stunting or wasting because of worm infestation (7). Undernutrition remains the world most serious health challenge and the single biggest contributor to child mortality (7).

Intestinal helminthic infections contribute to child undernutrition through subtle reduction in digestion and absorption of food, chronic inflammation and loss of nutrient, thus resulting in decreased immunity and increased susceptibility to infections (2,8). In addition, intestinal helminths secrete toxic bioactive compounds within the small intestine of the host, which deplete nutrients and impair absorption, thereby playing an important role in childhood malnutrition (9,10).

The 2018 National Nutrition and Health Survey (NNHS) showed that Nigeria accounts for 36% of malnourished children worldwide. However, the prevalence of malnutrition in Gombe State, Nigeria was 44% (11). This burden is likely to be increasing due to the activities of the terrorist organization "*Boko Haram*" which has led to displacement of people from Borno and Yobe to other north-eastern States especially Gombe State, thereby increasing the population of Gombe, leading to water scarcity and increased rate of infections, especially helminthic infections. This might further worsen the nutritional status of children in Gombe. There is therefore the need to improve the nutritional status of children in this State which has prompted the conduct of this study.

Information on the prevalence of helminthiasis will be necessary to provide appropriate authorities on the need to implement periodic deworming of primary school pupils in Gombe, as this may improve the nutritional status of pupils, to enable them attain their full potentials as adults. The objective of this study therefore is to determine the prevalence of intestinal helminthiasis among primary school pupils in Gombe LGA of Gombe State in Nigeria and to establish its association with nutritional status of the pupils.

Materials and method:

Study area:

The study was conducted in Gombe local government area (LGA), Gombe, the capital of Gombe State, located in the north-east geopolitical zone of Nigeria. Gombe LGA has 11 wards with a projected population of 280,000. There are 150 registered primary schools, of which 33 are government-aided and 117 are privately owned. Gombe has two distinct weather conditions; dry season (November to March) and the rainy season (April to October) with an average rainfall of 850mm (12). The indigenous people are mainly traders, farmers, artisans and civil servants, and the staple food in the State includes rice, maize and guinea corn.

Study design and subject participants:

This was a descriptive cross-sectional study of primary school children in Gombe LGA.

All primary school children whose parents/guardians gave informed consent, and children 7 years and above who gave informed assent in addition to consent were eligible and recruited for the study. Children with history suggestive of chronic illnesses such as HIV, diabetes mellitus and sickle cell anaemia were excluded.

Sample size estimation and sampling procedure:

The sample size of 375 was determined using the Fischer formula (13). A multi-stage random sampling technique was used to select the pupils. In the first stage, from the list of the 11 wards in Gombe LGA, 6 wards were selected by simple random sampling with balloting without replacement. The schools in the selected ward were stratified based on ownership into public and private. Three private and 1 public school were selected in the second stage from each selected ward using simple random sampling by balloting, which gave a total of 24 schools from the 6 selected wards.

In the third stage, the number recruited from each school and class was determined by the proportionate sampling method. After determining the number to be recruited from each class, the class register was used as a sampling frame and the pupils were selected by using systematic sampling technique. The sampling interval was determined by dividing the total number of pupils in each class by the number of pupils selected from the class. Pupils who did not fulfil the inclusion criteria were omitted and the next pupil on the list was selected. Thereafter, the sampling interval was used for selection of subsequent study participants.

Ethical consideration

Ethical approval was obtained from the Federal Teaching Hospital Gombe Research and Ethical Committee (NHREC/25/10/2013). A written permission was sought from the Education Secretary, Gombe State Universal Basic Education Board (SUBEB) and the management of the selected schools. Informed consent was obtained from the parents/guardians and pupils 7 years and above gave assent. The study was at no cost to the participants and all participants received albendazole 400mg stat.

Data and sample collection:

The study was conducted over a period of 7 months (July 2018-January 2019). A pilot study was carried out using 10% of the targeted sample size (38 participants) in a school that was not involved in the main study. This allowed the researcher to pre-test the research tools. No major changes were however made to the research materials following the pre-test study. The questionnaires were filled by the study subjects and their parents/guardians, and each subject was then examined for presence/absence of pallor and oedema. The weight and height of each pupil were subsequently measured.

Measurement of weight, height and calculation of body mass index:

The weight of each pupil was measured using a calibrated standardized digital weighing scale (OMRON BF400), with the accuracy of the scale set to the nearest 0.5 kg, with average of two readings taken. The height was measured using a wooden stadiometer placed on a flat surface to the nearest 0.1 cm at the eye level of the examiner. The BMI was calculated using the formula; BMI (Kg/m^2)=weight (Kg)/height (m^2), and the WHO growth chart was used to interpret the BMI results; with BMI below the 5^{th} percentile classified as underweight, 5^{th} to less than 85^{th} percentile as normal, 85^{th} to less than 95^{th} percentile as overweight, and 95^{th} and above as obesity (14).

Stool sample collection:

Stool samples were collected into clean polythene bag using a spatula (in a well-formed stool) or a spoon (in diarrheal stool) from where a small piece of stool was transferred into a well-labelled universal container containing information on subject age, gender and code number. The samples were transported within one hour of collection to the Research/Teaching Microbiology Laboratory, Gombe State University in a cold box and examined within one hour of collection.

Direct wet mount microscopy:

Direct wet mount was used to examine diarrheal stools. Using an applicator stick, a pinhead size of stool sample was applied to a small area on a clean glass slide and gross fibres and particles were removed immediately. Two drops of saline were added with a pipette and mixed with the tip of the applicator stick specimen was then covered with a cover slip. The cover-slip(s) was sealed using paraffin oil to prevent the preparation from drying out.

The slide was examined under a low-power microscope objective (10x). When a parasite-like object came into view, it was then examined and identified under high power (40x) objective of the microscope for ova or larvae of parasite (13). A total of 10 fields were examined in each slide for 10 minutes before a slide was declared to be negative. The medical microbiologist and the lead author examined the slides and where there was a discordance, a laboratory scientist served as the tie-breaker.

Formol-ether concentration method:

About 3g of faeces were suspended in 10 ml of 10% formol-saline (formalin in saline) solution and mixed with a glass rod. The suspension was sieved (0.5mm mesh sieve) into a centrifuge tube. Then 3ml of ether was added and the mixture was shaken vigorously before centrifuging for 5 minutes at 3,000 rpm, after which the supernatant was discarded leaving the sediment. A drop of the sediment was placed on a glass slide and covered with a cover slip. A drop of iodine added to the side of the cover slip then the deposit examined using 10x and 40x objectives of the microscope for ova or larvae. In each slide, 10 fields were examined for 10 to 15 minutes (15).

Stoll method for counting ova of parasites:

Ova of parasites was counted using the method of Stoll and Hausheer (16). Forty-two millilitres of water were measured into a dish, then a tongue depressor was used to push 3g of faeces through a sieve into the water, then the sieve was lifted above the dish to allow remaining water from the faeces to drip while stirring the water-faeces mixture. The suspension of 0.15ml spread over two slides; each slide was then covered with a long cover slip. Both slides were examined for worm eggs, the total number of eggs counted X 100 represents the number of eggs per gram of faeces. Since 0.15 ml is 1/300 of 45ml (42ml water and 3g faeces), the number of eggs in 0.15ml x 100 is equal to 1/3 of the total number of eggs in the original 3g and thus equal to eggs per gram (EPG).

Data analysis

All data generated were processed and analysed using the IBM Statistical Package for Social Sciences (IBM SPSS) software version 24.0. The means and standard deviations of quantitative variables were calculated while Chi-square test (with Odds ratio and 95% confidence interval) was used to determine association of nutritional status of participants with prevalence of helminthiasis. A $p<0.05$ was considered statistically significant

Results:

Three hundred and seventy-five pupils aged 5-14 years from 24 primary schools were recruited for the study but data of only 350 pupils were used for analysis due to missing and incomplete data. The mean age of the pupils was 9.51±2.20 years, and the male to female ratio was 1 to 1.2. One hundred and forty-seven (42%) are from low socio-economic status, and most of the pupils (233, 66.6%) were from public schools while 117 (33.4%) were from private schools (Table 1).

Prevalence and types of intestinal helminths isolated among the pupils:

Out of the 350 pupils, 83 were infected with helminths, giving a prevalence of 23.7%. Pupils in public schools accounted for 96.4% of the infections. Out of the 83 helminths identified, the most frequent was *Ascaris lumbricoides* (22, 26.5%) while the least frequent was *Trichuris trichuria* (2, 0.6%). Multiple helminths were identified in 15 (18.1%) subjects (Fig 1).

Nutritional status of the participants in Gombe using BMI:

The mean weight of the subjects was 25.6±7.32kg and mean height of 130±11.56 cm. Two hundred and six (58.9%) pupils had normal weight, 100 (28.6%) were underweight, 37 (10.6%) overweight and 7 (2.0%) were obese (Fig 2).

BMI in relation to sociodemographic characteristics of the study subjects

Table 2 shows bivariate analysis of the association between sociodemographic characteristics and body mass index (BMI) of the pupils. There was no significant association of underweight, normal weight, overweight and obesity with age groups, gender, and socio-economic status of the pupils ($p>0.05$), except for pupil in the high socio-economic class who had significantly higher prevalence of overweight (17.1%) than pupils in the middle (12.4%) and low socio-economic class (5.4%) ($p=0.0167$). The was statistically significant association between school types and underweight (OR=0.3943, $p=0.0012$), and school type and overweight (OR = 4.367, $p<0.0001$) (Table 2).

Table 1: Sociodemographic characteristics of the study participants

Characteristics	Frequency (n = 350)	Percentages (%)
School ownership		
Public	233	66.5
Private	117	33.4
Age group (years)		
5 - 9	156	44.6
10 -14	194	55.4
Gender		
Male	164	46.9
Female	186	53.1
Ethnicity/tribe		
Fulani	143	40.9
Hausa	82	23.4
Tera	30	8.6
Others	95	27.1
Socio economic class		
High	82	23.8
Middle	121	34.6
Low	147	42.0
Family type		
Monogamous	189	54
Polygamous	161	46
Number in household		
< 6	99	28.3
≥ 6	251	71.7

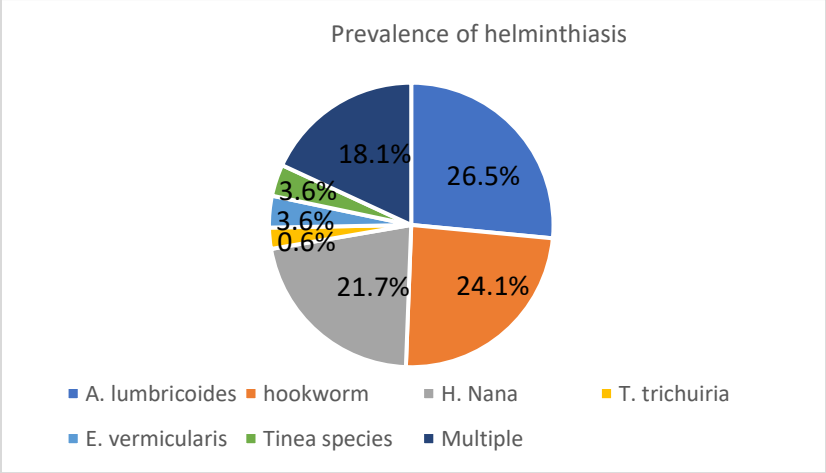

Fig 1: Pie chart showing prevalence and types of intestinal helminths isolated in the subjects

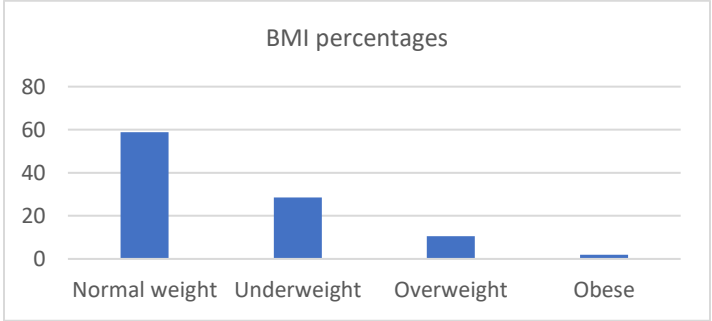

Fig 2: Bar chart showing BMI of the study subjects in percentages

Table 2: Bivariable analysis of sociodemographic characteristics in relation to body mass index of pupils in Gombe local government area, Gombe, Nigeria

Variable	Underweight	Normal weight	Over weight	Obese
Age group (years)				
5-9 years (n=156)	48 (30.8)	89 (57.1)	17 (10.9)	2 (1.3)
10-14 years (n=194)	52 (26.8)	117 (60.3)	20 (10.3)	5 (2.6)
x^2	0.4860	0.2564	$8.987E^{-06}$	0.2268
OR (95% CI)	1.214 (0.76-1.93)	0.8742 (0.57-1.34)	1.064 (0.54-2.11)	0.4909 (0.09-26)
P	0.4857	0.6126	0.9976	0.6339
Gender				
Male (n=164)	47 (28.7)	102 (62.2)	13 (7.9)	2 (1.2)
Female (n=186)	53 (28.5)	104 (55.9)	24 12.9)	5 (2.7)
x^2	0.001147	1.172	1.787	0.3562
OR (95% CI)	1.008 (0.63-1.61)	1.297 (0.85-1.99)	0.5811 (0.29-1.18)	0.4469 (0.09-0.23)
P	0.9730	0.2789	0.1813	0.5506
Socioeconomic class				
High (n=82)	18 (21.9)	47 (57.3)	14 (17.1)	3 (3.7)
Middle (n=121)	34 (28.1)	71 (58.7)	15 (12.4)	1 (0.8)
Low (n=147)	48 (32.7)	88 (59.9)	8 (5.4)	3 (2.0)
x^2	2.974	0.1435	8.184	2.002
p	0.2260	0.9308	0.0167*	0.3675
School type				
Private (n=117)	20 (17.1)	70 (59.8)	24 (20.5)	3 (2.6)
Public (n=233)	80 (34.3)	136 (58.4)	13 (5.6)	4 (1.7)
x^2	10.515	0.02152	16.827	0.01677
OR (95% CI)	0.3943 (0.23-0.69)	1.062 (0.68-1.67)	4.367 (2.13-8.95)	1.507 (0.33-0.69)
P	0.0012*	0.8834	<0.0001*	0.8790

*=statistically significant; OR-Odds ratio; CI-Confidence interval

BMI and adequacy of subject's diet:

Overweight was the only BMI category significantly associated with adequacy of 24-hour dietary intake, with inadequate dietary intake occurring significantly less frequently (24. 3%) among overweight pupils (OR=0.2959, *p*= 0.0025) while there was no significant association of dietary intake with other BMI categories (Table 3).

Association of intestinal helminthic infection and nutritional status of pupils:

The prevalence of helminthiasis was significantly higher (OR=2.113, *p*=0.0065) among underweight pupils (34%) and significantly lower (OR=0.1637, *p*=0.0037) among overweight pupils (5.4%) while the prevalence was not significantly associated with normal weight (*p*= 0.5482) or obesity (*p*=1.000) (Table 4).

Table 3: Bivariate analysis of the relationship of BMI with adequacy of typical 24-hour diet of pupils in Gombe local government area, Gombe, Nigeria

Variable	Inadequate diet n (%)	Adequate diet n (%)	x^2	OR (95% CI)	*p* value
Underweight	55 (55.0)	45 (45.0)	1.608	1.389 (0.8719-2.214)	0.2048
Normal weight	105 (51.0)	101 (49.0)	0.5035	1.195 (0.7799-1.830)	0.4780
Overweight	9 (24.3)	28 (75.7)	9.117	0.2958 (0.13-0.65)	0.0025*
Obese	3 (49.4)	4 (57.1)	0.1129	0.7722 (0.17-3.50)	0.7368
Total	**172 (49.1)**	**178 (50.9)**			

*=statistically significant; OR=Odds ratio; CI=confidence interval; x^2=Chi-square

Table 4: Bivariate analysis of the relationship between intestinal helminths and nutritional status of primary school pupils in Gombe local government area, Gombe, Nigeria

Variable	Infected n (%)	Uninfected n (%)	x^2	OR (95% CI)	*p* value
Underweight	34 (34.0)	66 (66.0)	7.411	2.113 (1.26-3.55)	0.0065*
Normal weight	46 (22.3)	160 (45.7)	0.3606	0.8314 (0.51-1.37)	0.5482
Over weight	2 (5.4)	35 (94.6)	6.577	0.1637 (0.04-0.69)	0.0037*
Obese	1 (14.3)	6 (85.7)	0.02063	0.5305 (0.06-4.47)	1.000
Total	**83 (23.7)**	**267 (76.3)**			

* Significant P value; OR=Odds ratio; CI=confidence interval; x^2=Chi-square

Discussion:

The prevalence of 23.7% for intestinal helminthic infections in primary school children in this study is high and similar to the reports by some researchers (17,18). Body mass index was used for nutritional assessment and underweight was used as a composite indicator to reflect both acute and chronic undernutrition in this study. Pupils with helminthiasis had lower BMI compared to those without helminthiasis, with 34.0% of the underweight pupils being infected while only 5.4% of overweight pupils had infection. This is similar to report by Oninla et al., (19) among infected and uninfected primary school pupils in Osun State, southwest Nigeria. Similar report was also documented from Calabar, south-east Nigeria where Meremikwu et al., (20) reported that greater proportion of underweight, stunted and wasted children had intestinal helminth infection compared to their well-nourished counterparts. Okolo et al., (21) in Vom, Plateau State and Quihui-Cota et al., (22) in Mexico have also reported high prevalence of intestinal helminths among undernourished children.

In contrast to the findings of the current study, Ojurongbe et al., (23) in Ile-Ife and Ajayi et al., (24) in Lagos reported no significant difference in the nutritional status of infected and uninfected primary school children, although the mean weight and BMI of the infected children was lower than uninfected children. The sample size used in the study by Ojurongbe et al., (23) was small and the methodology adopted in arriving at the sample size was not stated. This may have affected the statistical power of their study resulting in false negative findings or type II error. It was difficult to give plausible explanation to the findings by Ajayi et al., (24) as stated by the authors themselves. This was because the findings of the study by Ajayi et al., (24) contrasted many studies and contradicted the theory that proved the known relationship between intestinal helminth and nutrition.

The prevalence of both undernutrition and helminthic infections were significantly higher in pupils from public schools. This is because majority of the pupils were from low socioeconomic families and all the public schools had poor sanitary/toilet facilities, which could lead to contamination of the environment and therefore increasing the risk of helminthic infection. This is similar to the reports by Ogwurike et al., (25) and Obiukwu et al., (26). Undernutrition has been shown to increase susceptibility to helminthic infections, which in turn impair the nutritional status of the host (27). Geographical clustering of helminthic infections, malnutrition and poverty has also been demonstrated similar to the finding of current study (28).

Conclusion:

The prevalence of intestinal helminthic infection among primary school children aged 5-14 years in Gombe LGA, Gombe State, Nigeria in our study was 23.7%, and majority of the infections were in children from public schools who belonged to low socioeconomic class. Under nutrition was significantly associated with intestinal helminthic infection in the pupils especially those in public schools. Therefore, provision of toilet facilities and clean water with regular provision of free antihelminth medications especially in public schools are recommended.

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Contributions of authors:

HBB conceived the study idea and design, led the data collection, conducted analysis, drafted and critically reviewed and revised the manuscript; RA contributed to the study design, manuscript draft, critical review and revision of the manuscript; MMM contributed to the study design, critical review and revision of the manuscript; WSB and UAS contributed to analysis, critical review and revision of the manuscript.

Conflict of interest:

No conflict of interest is declared

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Assessment of faecal contamination in selected concrete and earthen ponds stocked with African catfish, *Clarias gariepinus*

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Abstract:

Background: Microorganisms constitute significant fraction of the aquatic ecosystem and have been reported to be the cause of emerging novel infectious diseases in aquacultural practices. The prevalence of infectious diseases has been observed to depend on the interaction between fish pathogens and the aquatic environment. This study was conducted to assess the levels of faecal pollution markers in catfish (*Clarias gariepinus*) and their growing waters in selected earthen and concrete ponds in the teaching and research fish farm of the Federal University of Technology, Akure (FUTA), Nigeria in the dry (February-April) and wet seasons (May-July) of the year.
Methodology: Two earthen and 2 concrete ponds were randomly selected as sampling sites due to their frequent usage. A total of 120 grabs of catfishes from the earthen (n=60) and concrete (n=60) ponds, and 84 pond water samples from earthen (n=42) and concrete (n=42) ponds, were randomly collected over a 6-month period of study. Enteric bacteria count in the water and catfish samples were determined using membrane filtration and pour plate methods respectively. The physiochemical characteristics of the water samples were determined using standard methods. The rate of bioaccumulation of faecal indicator bacteria was obtained by dividing the log count of each organism in the catfish by the corresponding log count in the growing waters.
Results: Faecal coliforms count (log_{10} CFU/100ml) in the catfish from concrete and earthen ponds ranged from 1.41 to 2.28 and 1.3 to 2.47, and in the growing waters; 1.43 to 2.41 and 1.50 to 2.80 respectively. There was positive correlation of faecal coliforms with alkalinity of water samples from the earthen (r=0.61) and concrete ponds (r=0.62). *Salmonella* and faecal coliforms had the highest and least bioaccumulation in catfish raised in earthen pond while *Salmonella* and enterococci had the highest and least bioaccumulation in catfish raised in concrete pond respectively. Faecal coliforms and *Escherichia coli* had the highest and least counts in water samples from the earthen pond during the dry and wet months while *Salmonella* and *E. coli* had the highest and least counts in water samples from the concrete pond during the dry and wet months.
Conclusion: High levels of bacterial faecal pollution markers in water samples and catfishes from the earthen and concrete ponds are reported in this study. Physicochemical characteristics and seasonality played major roles in the rate of bioaccumulation of the faecal pollution markers in catfishes raised in both earthen and concrete ponds.

Keywords: Bioaccumulation; *Clarias gariepinus;* earthen; concrete; pond; coliforms; seasonality

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Évaluation de la contamination fécale dans des étangs en béton et en terre sélectionnés remplis de poisson-chat Africain, *Clarias gariepinus*

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Résumé:

Contexte: Les micro-organismes constituent une fraction importante de l'écosystème aquatique et ont été signalés comme étant la cause de nouvelles maladies infectieuses émergentes dans les pratiques aquacoles. Il a été observé

que la prévalence des maladies infectieuses dépend de l'interaction entre les agents pathogènes des poissons et l'environnement aquatique. Cette étude a été menée pour évaluer les niveaux de marqueurs de pollution fécale chez le poisson-chat (*Clarias gariepinus)* et leurs eaux de croissance dans des étangs en terre et en béton sélectionnés dans la pisciculture d'enseignement et de recherche de l'Université fédérale de technologie d'Akure (FUTA), au Nigeria, dans le saisons sèches (Février - Avril) et humides (Mai - Juillet) de l'année.

Méthodologie: Deux étangs en terre et 2 en béton ont été choisis au hasard comme sites d'échantillonnage en raison de leur utilisation fréquente. Un total de 120 prises de poissons-chats des étangs en terre (n=60) et en béton (n=60), et 84 échantillons d'eau des étangs en terre (n=42) et en béton (n=42), ont été prélevés au hasard sur une période d'études de 6 mois. Le nombre de bactéries entériques dans les échantillons d'eau et de poisson-chat a été déterminé en utilisant respectivement les méthodes de filtration sur membrane et de plaque de coulée. Les caractéristiques physicochimiques des échantillons d'eau ont été déterminées à l'aide de méthodes standard. Le taux de bioaccumulation des bactéries fécales indicatrices a été obtenu en divisant le nombre logarithmique de chaque organisme dans le poisson-chat par le nombre logarithmique correspondant dans les eaux de croissance.

Résultats: Le nombre de coliformes fécaux (log_{10} UFC/100ml) chez le poisson-chat des étangs en béton et en terre variait de 1,41 à 2,28 et de 1,3 à 2,47, et dans les eaux de croissance; 1,43 à 2,41 et 1,50 à 2,80 respectivement. Il y avait une corrélation positive des coliformes fécaux avec l'alcalinité des échantillons d'eau des étangs en terr (r=0,61) et en béton (r=0,62). Les *Salmonella* et les coliformes fécaux présentaient la bioaccumulation la plus élevée et la plus faible chez les poissons-chats élevés dans des étangs en terre, tandis que les *Salmonella* et les entérocoques présentaient respectivement la bioaccumulation la plus élevée et la plus faible chez les poissons-chats élevés dans des étangs en béton. Les coliformes fécaux et *Escherichia coli* présentaient les taux les plus élevés et les moins élevés dans les échantillons d'eau de l'étang en terre pendant les mois secs et humides, tandis que *Salmonella* et *E. coli* avaient les taux les plus élevés et les moins élevés dans les échantillons d'eau de l'étang en béton pendant les mois secs et humides.

Conclusion: Des niveaux élevés de marqueurs de pollution fécale bactérienne dans les échantillons d'eau et les poissons-chats des étangs en terre et en béton sont rapportés dans cette étude. Les caractéristiques physicochimiques et la saisonnalité ont joué un rôle majeur dans le taux de bioaccumulation des marqueurs de pollution fécale chez les poissons-chats élevés dans des étangs en terre et en béton.

Mots clés: Bioaccumulation; *Clarias gariepinus*; en terre; béton; étang; coliformes; saisonnalité

Introduction:

Within the genus Clarias, *Clarias gariepinus* is one of the most researched tropical catfish (1). Aquaculture has received a lot of attention recently as a fast-growing sector of global food production and a source of animal protein. Microorganisms have a key role in the aquatic ecosystem, and have been identified as one of the variables that can lead to outbreaks of infectious diseases in aquaculture (2). The interplay between fish pathogens and the aquatic environment has been shown to influence the occurrence of infectious illnesses (2). As a result, there is a need to quantify and track the microbial population in this industry.

The African catfish has been imported into at least 37 African, European, Asian, and American countries, primarily for aquaculture with economic consequences on freshwater and brackish ecosystem (3). The species have been introduced to the Caribbean for aquaculture in Cuba (4). *Clarias gariepinus* has evolved important adaptations for surviving in unsuitable environments with low oxygen levels and long periods of desiccation (5,6). According to Omeji et al., (7), water polluted with bacteria (faecal coliforms, faecal streptococci, and *Salmonella* spp) could infect fish in ponds. Bacteria can be found on the surface of decomposing materials such as leaves, metallic objects, rocks, and wood in earthen ponds. The distribution of heterotrophic bacteria and total aquatic bacteria in the earthen pond varies according to the water layers. The circulation of bacteria in fish pond, which includes predatory protozoa present in the water, is influenced by a variety of circumstances (8).

Bacteriological study of fish pond water is critical in aquaculture because it might reveal potential risks to the fish, the farmers and customers (8). Allowing cattle to graze near water bodies, spreading manure as fertilizer on fields during wet periods, employing sewage sludge bio-solids, and allowing livestock to drink from streams have all been linked to faecal contamination of ponds (9). Because majority of faecal pollution comes from non-point and multiple extended sources, preventing excessive faecal contamination of ponds is difficult (10). Faeces from poultry fowl are directly released into some integrated ponds which permit the development of maggots that are then discharged into the ponds.

Microorganisms, particularly those of coliform category, have been found in fish and their aquatic habitats. Furthermore, pond water contaminated with faeces poses a major risk to human health when released into other bodies of water (11). The aim of this study is to track faecal pollution markers in catfish (*Clarias gariepinus*) and their growing waters in varieties of earthen and concrete ponds. The objectives of the study are; (i) to determine the counts of faecal indicator bacteria (FIB) in catfish and their growing waters; (ii) assess the physico-

chemical characteristics of the growing waters and determine their relationship with FIB; (iii) determine the rates of FIB bioaccumulation in catfish; and (iv) investigate the effect of different seasons on FIB bioaccumulation in catfish.

Materials and method:

Study setting and selection of sampling sites:

The study setting is the Teaching and Research Fish Farm, Obakekere campus of the Federal University of Technology Akure (FUTA), Nigeria, located between latitude 7° and 8°N and longitude 5° and 18°E with elevation of approximately 30.5 metres (100 feet) above sea level (Fig 1). The area contains characteristic swamp, water ways, vast plains and rain forest. The farm combines fish production and livestock production, and also operates integrated fish production with different concrete and earthen ponds stocked with tilapia, catfish and other species of fish.

Two each of the earthen and concrete ponds were randomly selected as the sampling sites because of the frequent usage of these ponds. The ponds are also prone to faecal contamination from farm animals grazing around the ponds, and the landscape encourages direct flow of erosion waters into the earthen pond during wet season.

Study design:

This was a descriptive cross-sectional study of catfishes and their growing waters from earthen and concrete ponds in fish farms of Federal University of Technology Akure (FU TA), Nigeria, conducted over a period of 6 months (February to July 2019). The schematic flowchart of the study is as shown in Fig 2.

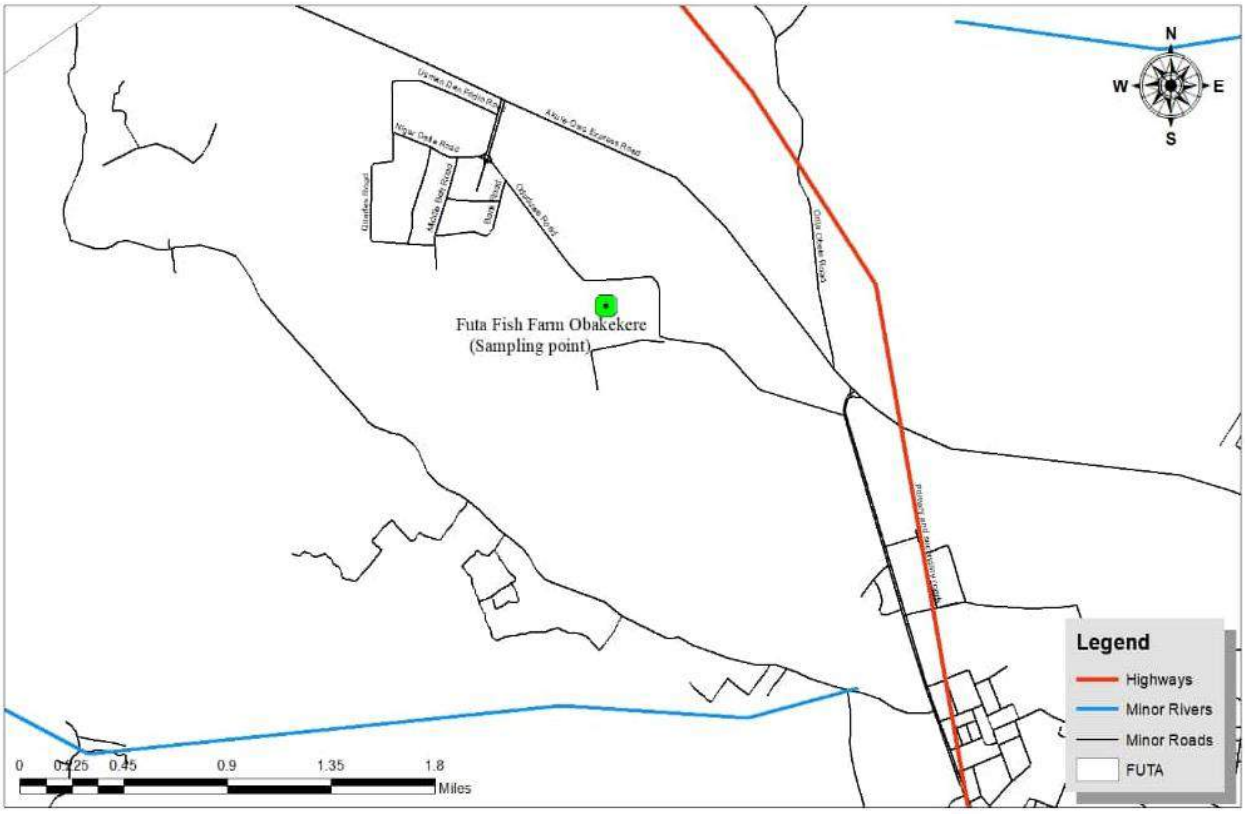


Fig 1: Map of Teaching and Research Fish Farm in Obakekere campus, FUTA, Nigeria

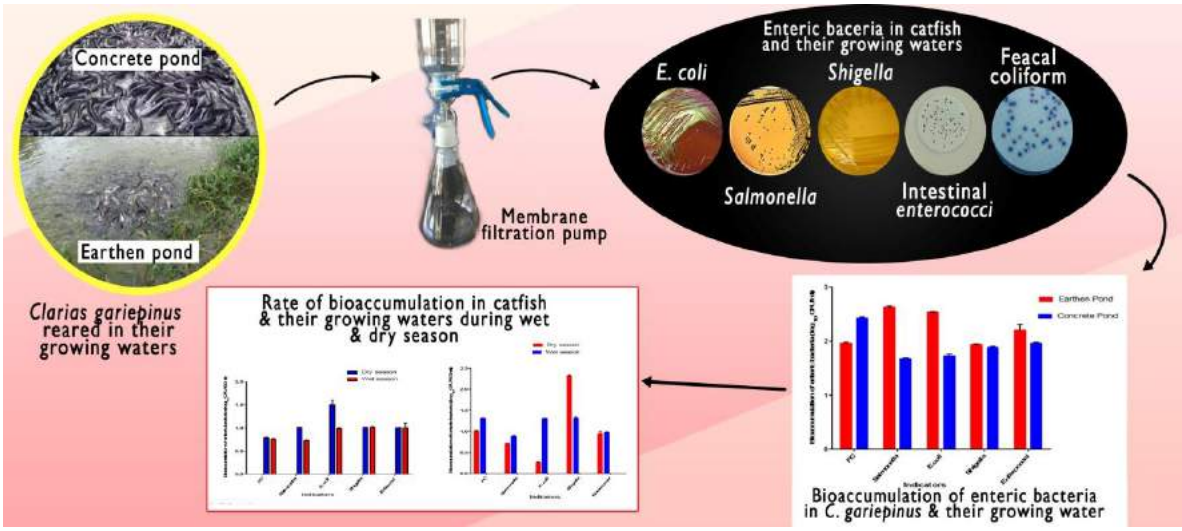


Fig 2: Schematic illustration for the assessment of faecal contamination in selected concrete and earthen ponds stocked with *Clarias gariepinus*

Collection of catfish and growing water samples from the ponds:

Water and catfish samples were collected weekly from February to July 2019. On each sampling occasion, water and catfish samples were collected from two earthen and two concrete ponds in a fish farm. The water samples were collected aseptically with sterile 800 ml screw-capped bottles labeled appropriately and the catfish samples were collected with conventional scoop net and thereafter placed in a sterile polythene bag with appropriate labeling. In total, 84 grabs of water and 120 catfish samples were collected over the study period through standard protocols. All samples were transported to the laboratory within 1 hour.

Enumeration of enteric bacteria in catfish and growing water samples:

The counts of *Escherichia coli*, faecal coliforms, *Salmonella, Shigella* and intestinal enterococci in the catfish and their growing waters were determined using standard microbiological methods as described by Maheux et al., (12). Preparation of the catfish samples was carried out by dissecting the intestinal tract of the fish using a sterile knife and measuring 1g into a sterile mortar. This was macerated with about 4ml of sterile distilled water and 1ml aliquot was taken into a sterile test tube containing 9ml of sterile distilled water resulting into 1:10 dilution. Serial dilution was carried out until the fifth dilution.

Using membrane filters (0.45µm), the bacteria counts were determined by placing the filters on freshly prepared selective media; membrane lauryl sulphate agar (MLSA), eosin methylene blue (EMB), membrane faecal coliform agar (m-FC), membrane intestinal enterococci agar (m-EA) and *Salmonella–Shigella* agar (SSA). Agar plates were incubated at 37ºC for 24 hours (for MLSA, EMB and SSA), 44ºC for 24 hours (for m-FC) and 37ºC for 48 hours (for m-EA). Colonies were counted and expressed as colony forming unit (CFU) per 100ml of water or CFU per 100g of catfish using a colony counter (J-2 PEC MEDICAL, New Jersey, USA).

Determination of the physicochemical characteristics of growing waters in the ponds:

The temperature of the growing water was determined on-site during sample collection using the mercury-in-glass thermometer (ACCU-SAFE ThermoScientific, New Jersey, US). The pH, electrical conductivity, salinity, total dissolved solids, turbidity and dissolved oxygen of the water samples were determined using a multi-parameter analyzer (HI98194, PH/ORP/EC/DO). The biological oxygen demand (BOD) was determined by the Winkler's method and the chemical oxygen demand (COD) of the water samples was determined by the method described by Kolb et al., (13).

Bioaccumulation of enteric bacteria in catfish samples from the ponds:

The rate of bioaccumulation of faecal indicator bacteria was obtained by dividing the log count of each organism in catfish by the corresponding log count in the growing waters at the same point in time.

Statistical analysis:

Data were transformed into $\log_{10}$ and examined using general descriptive statistics.

The normality and distribution pattern of the enteric bacteria in the catfish and their growing waters in concrete and earthen ponds were determined using Kolmogorov-Smirnov and Shapiro-Wilk statistics. Further analyses were carried out using one-way analysis of variance (ANOVA) with significance at $p<0.05$ on GraphPad Prism version 5.0 for faecal indicator bacteria counts (mean ± standard error), and physicochemical characteristics (mean ± standard deviation) of growing water in the concrete and earthen ponds. The relationships between enteric bacteria counts and physicochemical properties of the growing waters from the earthen and concrete ponds were analyzed using the Pearson's correlation coefficient at $p<0.05$ level of significance.

Results:

Enteric bacterial counts in catfish samples and their growing waters in the ponds:

The faecal coliform counts (log_{10} CFU/ml) in catfish samples from the concrete and earthen ponds ranged from 1.41 to 2.28 and 1.3 to 2.47 respectively, while the counts ranged from 1.43 to 2.41 and 1.50 to 2.80 in the growing water samples from the concrete and earthen ponds respectively (Fig 3). In catfish samples from concrete and earthen ponds, *Salmonella* counts (log_{10} CFU/ml ranged from 1.52 to 2.56 and 1.60 to 2.70 respectively, while in the growing water samples, the counts ranged from 1.51 to 2.56 and 1.60 to 2.70 respectively (Fig 4).

Escherichia coli counts (log_{10} CFU/100 ml) in catfish samples from the concrete and earthen ponds ranged from 1.23 to 2.44 and 1.30 to 2.30 respectively, while the counts in the growing water samples from the concrete and earthen ponds ranged from 1.30 to 2.1 and 1.20 to 1.53 respectively (Fig 5). *Shigella* counts (log_{10} CFU/ml) in catfish samples from concrete and earthen ponds ranged from 1.0 to 1.2 and 1.40 to 1.50 respectively while the counts in growing water samples ranged from 1.46 to 1.60 and 1.40 to 1.50 respectively (Fig 6). Enterococci counts (log_{10} CFU/ml) in catfish samples from concrete and earthen ponds ranged from 1.30 to 1.40 and 1.30 to 1.70 respectively, while the counts in growing water samples ranged from 1.40 to 1.50 and 1.32 to 1.82 respectively (Fig 7).

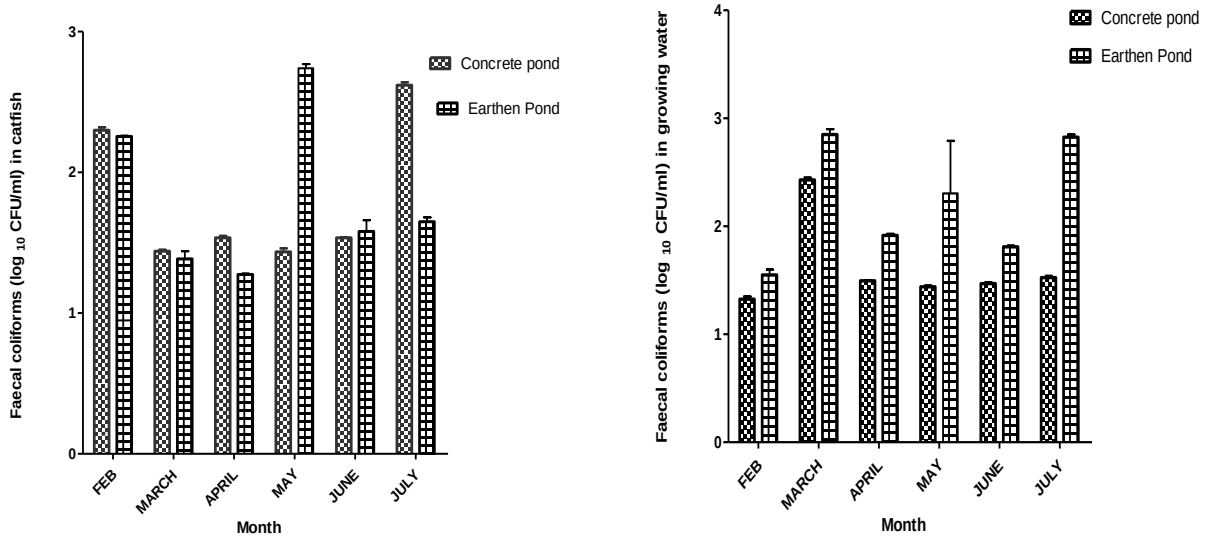


Fig 3: Mean concentration of faecal coliforms in catfish and their growing water samples in concrete and earthen ponds. Values are expressed as mean ± standard error of mean (SEM) of log_{10} CFU/100 ml

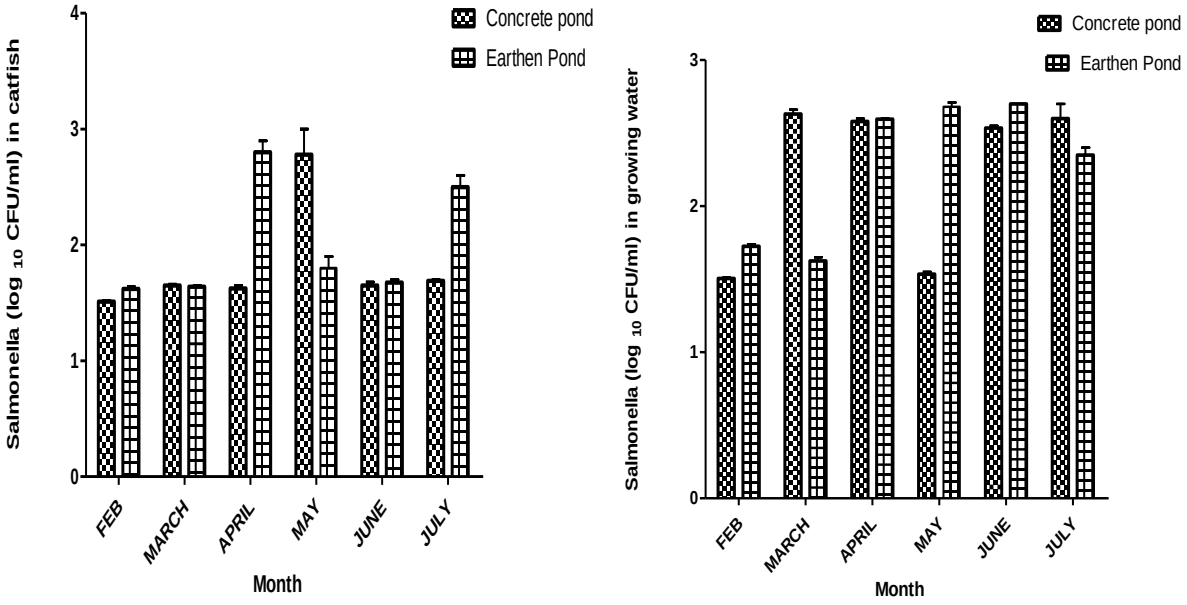


Fig 4: Mean concentration of *Salmonella* in catfish and their growing water samples in concrete and earthen pond. Values are expressed as mean ± standard error of mean (SEM) of log_{10} CFU/100 ml

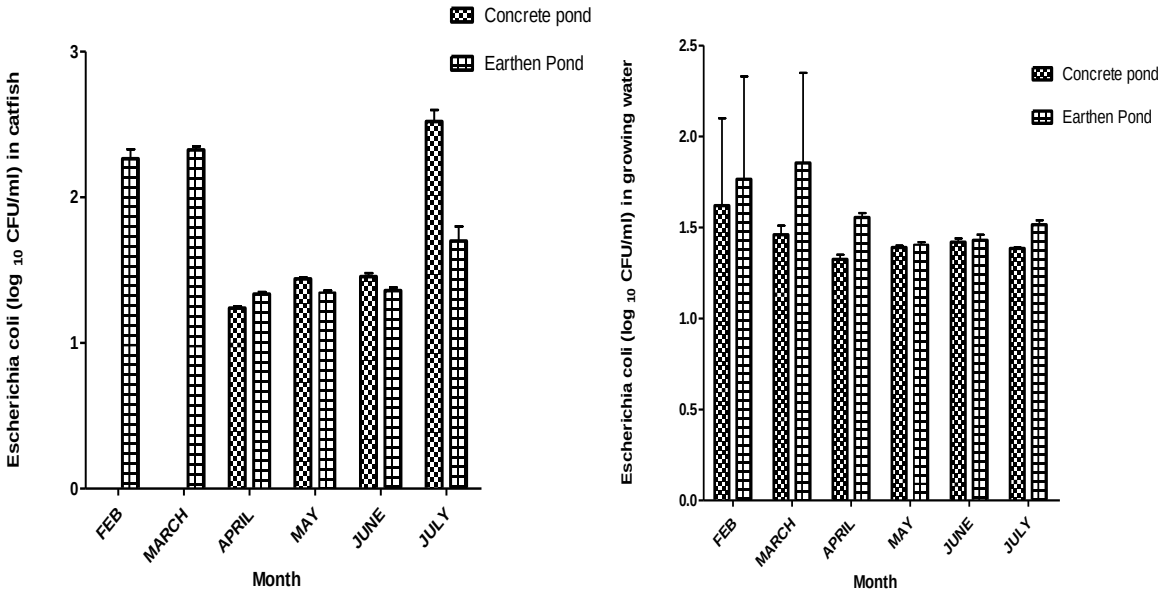


Fig. 5: Mean concentration of *E. coli* in catfish and their growing water samples in concrete and earthen ponds. Values are expressed as mean ± standard error of mean (SEM) of log_{10} CFU/100 ml

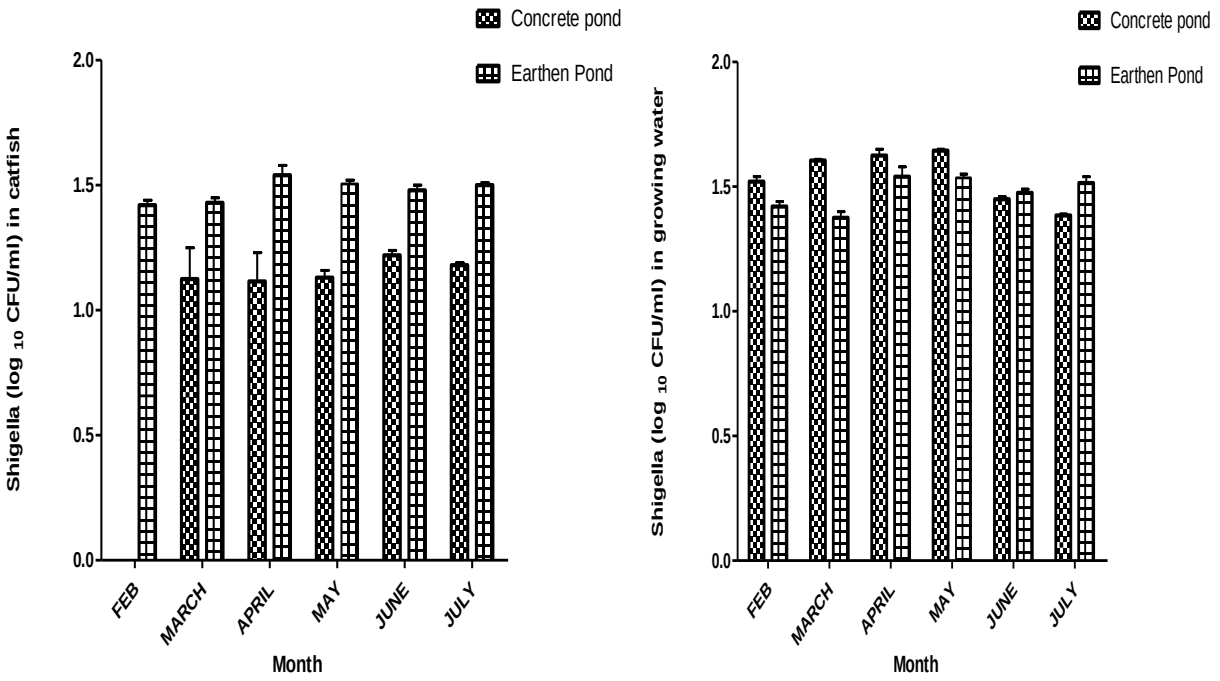


Fig 6: Mean concentration of *Shigella* in catfish and their growing water samples in concrete and earthen ponds. Values are expressed as mean ± standard error of mean (SEM) of log_{10} CFU/100 ml

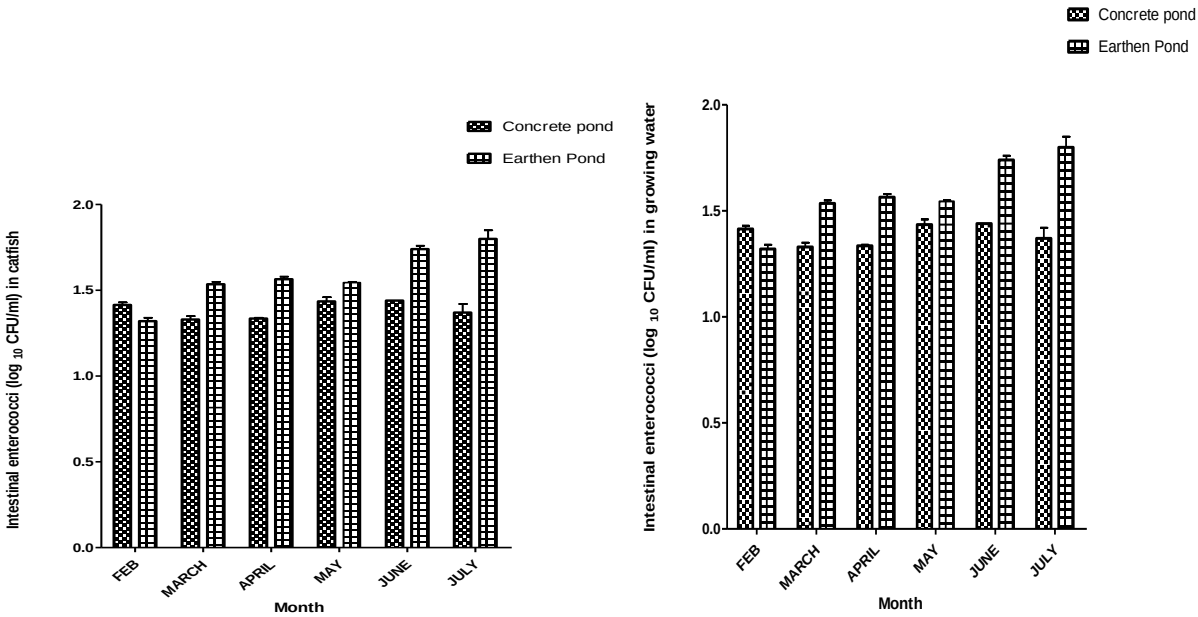


Fig. 7: Mean concentration of intestinal enterococci in catfish and their growing water samples in concrete and earthen ponds. Values are expressed as mean ± standard error of mean (SEM) of log_{10} CFU/100 ml

Distribution pattern of enteric bacteria in catfish and growing waters from the ponds:

The frequency distribution patterns of the enteric bacteria showed that faecal coliforms (Sig.=0.757) and *Salmonella* (Sig.= 0.843) in catfish samples from the earthen ponds were normally distributed while enterococci (Sig.= 0.001), *Shigella* (Sig.=0.029) and *E. coli* (Sig.= 0.028) were not normally distributed. Furthermore, *Salmonella* (Sig.=0.761) and *Shigella* (Sig.=0.761) counts in water samples from the earthen pond were normally distributed, while faecal coliforms (Sig.=0.000), enterococci (Sig. =0.038) and *E. coli* (Sig.=0.038) were not normally distributed (Table 1).

On the other hand, faecal coliforms (Sig.=0.527) and *Salmonella* (Sig.=0.643) in catfish samples from the concrete ponds were

normally distributed while enterococci (Sig.= 0.011), *Shigella* (Sig.=0.029) and *E. coli* (Sig. =0.018) significantly deviated from normal distribution. In addition, *Salmonella* (Sig.=0.461) and *Shigella* (Sig. =0.751) in water samples from the concrete ponds were normally distributed while faecal coliforms (Sig.=0.000), enterococci (Sig.=0.038) and *E. coli* (Sig.=0.028) significantly deviated from normal distribution (Table 2).

Table 1: Normality and distribution pattern of enteric bacteria in *Clarias gariepinus* and their growing water in earthen ponds

Test of Normality	**Kolmogorov-Smirnov[a]**			**Shapiro-Wilk**		
	Statistic	**Df**	**Sig.**	**Statistic**	**Df**	**Sig.**
Catfish samples						
Faecal coliforms	0.130	20	0.200*	0.970	20	0.757
Salmonella	0.120	20	0.200*	0.974	20	0.843
Enterococci	0.258	20	0.001	0.795	20	0.001
Shigella	0.197	20	0.040	0.891	20	0.029
Escherichia coli	0.141	20	0.200*	0.891	20	0.028
Water samples						
Faecal coliforms	0.345	20	0	0.717	20	0
Salmonella	0.096	20	0.200*	0.970	20	0.761
Enterococci	0.208	20	0.024	0.898	20	0.038
Shigella	0.096	20	0.200*	0.970	20	0.761
Escherichia coli	0.208	20	0.024	0.898	20	0.038

Df = Difference; Sig. = Significance

Table 2: Normality and distribution pattern of enteric bacteria in *Clarias gariepinus* and their growing water in concrete pond

Tests of Normality	**Kolmogorov-Smirnov[a]**			**Shapiro-Wilk**		
	Statistic	**Df**	**Sig.**	**Statistic**	**Df**	**Sig.**
Catfish samples						
Faecal coliforms	0.130	20	0.200*	0.970	20	0.757
Salmonella	0.120	20	0.200*	0.974	20	0.843
Enterococci	0.258	20	0.001	0.795	20	0.001
Shigella	0.197	20	0.040	0.891	20	0.029
Escherichia coli	0.141	20	0.200*	0.891	20	0.028
Water samples						
Faecal coliforms	0.345	20	0	0.717	20	0
Salmonella	0.096	20	0.200*	0.970	20	0.761
Enterococci	0.208	20	0.024	0.898	20	0.038
Shigella	0.096	20	0.200*	0.970	20	0.761
Escherichia coli	0.208	20	0.024	0.898	20	0.038

Df = Difference; Sig. = Significance

Table 3: Physicochemical characteristics of growing water samples in earthen and concrete ponds

Parameters	**Earthen pond**	**Concrete pond**
Temperature (°C)	26.7±0.28	29.3±0.35
pH	7.0±0.19	6.95±0.07
EC (µs/cm)	20.8±1.06	26.8±0.35
Salinity (PSU)	149.3±1.06	148.0±0.71
Turbidity (NTU)	43.5±0.25	44.3±0.29
TDS (mg/L)	23.5±0.38	22.3±0.35
Alkalinity (meq/L)	109.8±2.89	107.8±3.89
DO (mg/L)	11.95±2.13	14.6±6.29
BOD (mg/L)	2.53±1.23	1.53±0.03
COD (mg/L)	199.6±2.55	202.5±3.54

Values presented are expressed as mean values ± standard deviation (n=6). Temp = Temperature; EC = Electrical conductivity; TDS = Total dissolved solids; DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand.

Physicochemical characteristics of the growing waters in the earthen and concrete ponds:

In the earthen and concrete ponds, the mean temperatures of the growing waters were 26.70 ± 0.28°C and 29.30 ± 35.5°C respectively (Table 3). In the earthen and concrete ponds, the mean salinity of the growing waters was 149.31.06 PSU (practical salinity unit) and 148.00.71 PSU (practical salinity unit), respectively. Furthermore, the mean values of turbidity of the growing waters in the earthen and concrete ponds were 43 and 44 (NTU) (Nephelometric turbidity unit), respectively, whereas the mean values of total dissolved solids of the growing waters in the earthen and concrete ponds were 23 and 22 mg/L. The alkalinity levels of the growing waters in the earthen and concrete ponds were respectively 109 and 107 meq/L.

Relationship between enteric bacteria in catfish and physicochemical characteristics of the growing waters in the ponds:

In the earthen pond, alkalinity correlated positively with enterococci (r=0.600, p< 0.05), faecal coliforms (r=0.610, p<0.05), and *E. coli* counts (r=0.650, p<0.01). The total dissolved solids positively correlated with *E. coli* (r=0.51, p<0.05). Turbidity showed a positive correlation with faecal coliforms (r=0.54, p< 0.05). Biological oxygen demand had positive correlation with enterococci (r=0.52, p<0.05) and *E. coli* (r=0.75, p<0.01). Chemical oxygen demand also positively correlated with enterococci (r=0.500, p<0.05), faecal coliforms (r= 0.530, p<0.05), and *E. coli* (r=0.750, p<0.01) (Table 4).

In the concrete ponds, alkalinity had a positive correlation with faecal coliforms (r= 0.620, p<0.01) and *E. coli* (r=0.600, p<0.01). Total dissolved solids had a positive correlation with *E. coli* (r=0.650, p<0.05). Biological oxygen demand had a positive correlation with enterococci (r=0.580, p<0.05) and *E. coli* (r= 0.820, p<0.01). Chemical oxygen demand had positive correlation with enterococci (r=0.540, p<0.05) and faecal coliforms (r=0.580, p< 0.05). Salinity had a positive correlation with enterococci (r=0.51, p<0.05) and *E. coli* (r= 0.50, p<0.05) (Table 5).

Table 4: Correlation of enteric bacteria and physicochemical characteristics of growing waters in earthen pond

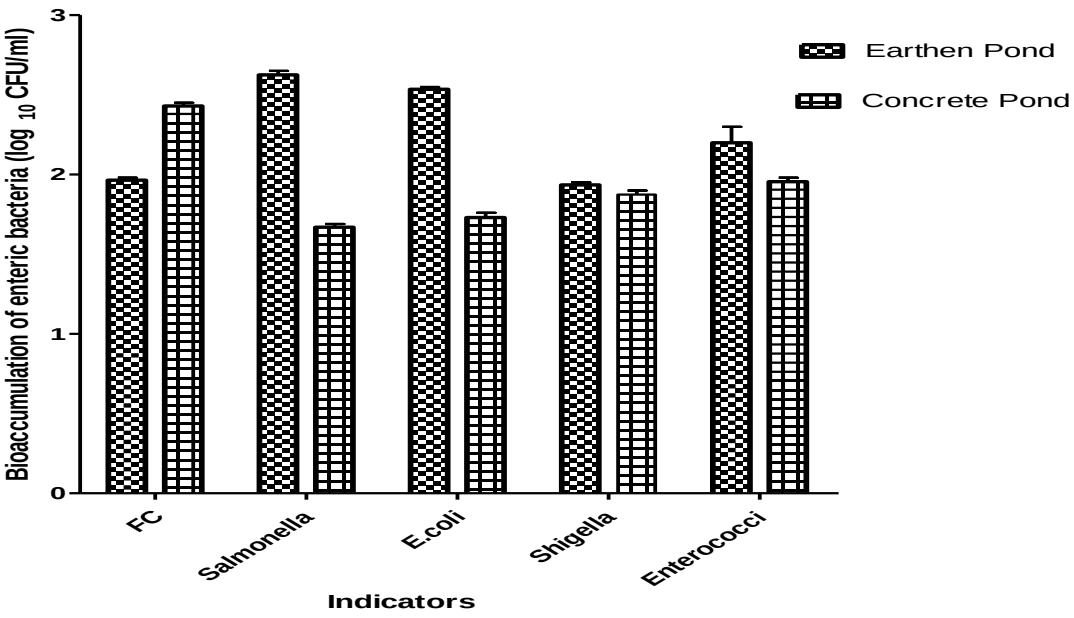
Physicochemical parameters	Enterococci	Faecal coliforms	*Escherichia coli*	*Salmonella*	*Shigella*
Temp (°C)	0.03	0.04	0.08	-0.10	-0.05
pH	-0.32	0.08	0.05	-0.28	-0.29
EC (µs/cm)	0.05	0.43	0.08	0.48	0.49
Turbidity (NTU)	0.10	**0.54***	0.34	0.29	0.22
Alkalinity (meq/L)	**0.60**	**0.61**	**0.65**	-0.18	-0.08
DO (mg/l)	-0.10	-0.13	0.29	-0.02	-0.01
Salinity (PSU)	0.31	-0.03	0.30	0.16	0.16
TDS (mg/L)	0.34	0.11	**0.51***	-0.14	-0.14
BOD (mg/L)	**0.52***	0.44	**0.75**	-0.18	-0.18
COD (mg/L)	**0.50***	**0.53***	**0.73**	-0.14	-0.14

Correlation is significant at the 0.05 level (2-tailed) *; Correlation is significant at the 0.01 level (2-tailed) **; Temp = Temperature; EC=Electrical conductivity; DO=Dissolved oxygen; TDS= Total dissolved solids; BOD=Biological oxygen demand; COD=Chemical oxygen demand.
Values in bold figures indicate significant correlation

Table 5: Correlation of enteric bacteria and physicochemical characteristics of growing waters in concrete pond

Physicochemical parameters	Enterococci	Faecal coliforms	*Escherichia coli*	*Salmonella*	*Shigella*
Temp (°C)	-0.11	-0.20	-0.22	-0.11	-0.12
pH	-0.42	-0.08	-0.15	-0.25	-0.25
EC (µs/cm)	-0.05	0.33	0.04	0.29	0.29
Turbidity (NTU)	0.10	0.37	0.21	0.19	0.19
Alkalinity (meq/L)	0.45	**0.62**	**0.60**	-0.28	-0.28
DO (mg/l)	-0.16	-0.23	0.19	-0.04	-0.04
Salinity (PSU)	**0.51***	0.03	**0.50***	0.36	0.26
TDS (mg/l)	0.44	0.21	**0.65**	0.18	-0.10
BOD (mg/l)	**0.58***	0.49	**0.82**	0.19	-0.12
COD (mg/l)	**0.54***	**0.53***	**0.77**	0.04	0.04

Correlation is significant at the 0.05 level (2-tailed) *; Correlation is significant at the 0.01 level (2-tailed) **; Temp=Temperature; EC=Electrical conductivity; DO=Dissolved oxygen; TDS=Total dissolved solids; BOD=Biological oxygen demand; COD=Chemical oxygen demand.
Values in bold figures indicate significant correlation



FC- Faecal coliforms

Fig 8: The bioaccumulation of enteric bacteria in catfish from their growing waters in earthen and concrete ponds

Bioaccumulation of enteric bacteria in catfish samples from the ponds:

Enterococci bioaccumulation (log_{10} CFU /ml) in catfish from their growing waters in earthen and concrete ponds ranged from 0.90 to 2.20 and 0.92 to 2.19 respectively. Faecal coliform bioaccumulation in catfish from earthen and concrete ponds ranged from 0.79 to 2.00 and 0.70 to 2.40 respectively. The bioaccumulation of enteric bacteria in catfish reared in concrete pond had a positive correlation with alkalinity, total dissolved solids, biological oxygen demand, chemical oxygen demand and salinity, and a negative correlation with temperature, pH, dissolved oxygen, and turbidity.

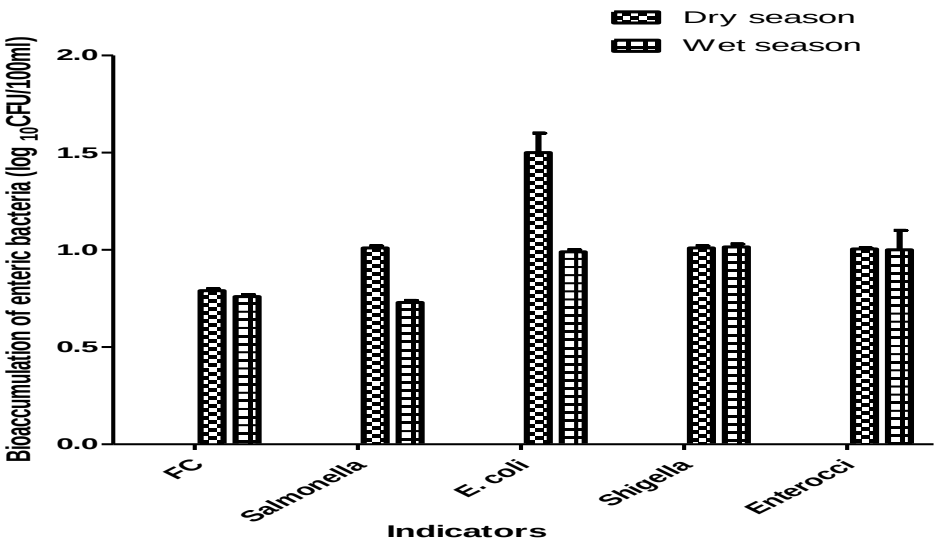
Escherichia coli bioaccumulation (log_{10} CFU/ml) in catfish from their growing waters in earthen and concrete ponds ranged from 0.94 to 2.65 and 0.00 to 1.89 respectively, while *Salmonella* bioaccumulation in catfish from their growing waters in earthen and concrete ponds ranged from 0.97 to 2.70 and 0.95 to 1.75 respectively. In earthen ponds, alkalinity, total dissolved solids, turbidity, biological oxygen demand, and chemical oxygen demand all had positive correlation with enteric bacteria in cat fish with the exception of *Salmonella* and *Shigella*, while salinity, temperature, pH, electrical conductivity, and dissolved oxygen had negative correlation. Furthermore, *Shigella* bioaccumulation (log_{10} CFU/ml) in catfish from their growing waters in earthen and concrete ponds was 0.97 to 1.89 and 0.95 to 1.86 respectively (Fig 8).

Effects of seasonality on bioaccumulation of enteric bacteria in catfish from their growing waters:

In the earthen ponds, the mean values of bioaccumulation (log_{10} CFU/ml) of enterococci in catfish samples in dry and wet periods were 0.99 and 0.62 respectively, while the mean values of bioaccumulation of faecal coliforms in catfish samples in dry (February - April) and wet periods (May - July) were 0.50 and 0.51 respectively. The mean values of bioaccumulation (log_{10} CFU/100ml) of *E. coli* in catfish samples in dry and wet periods were 1.02 and 0.89 respectively. The mean values of bioaccumulation (log_{10} CFU/100ml) of *Salmonella* in catfish samples in dry and wet periods were 0.60 and 0.45 respectively while the mean values of bioaccumulation of *Shigella* in catfish samples in dry and wet periods were 0.55 and 0.54 respectively. *Escherichia coli* had the highest mean value of bioaccumulation during the dry period while *Shigella* had the highest mean value of bioaccumulation during the wet period (Fig 9).

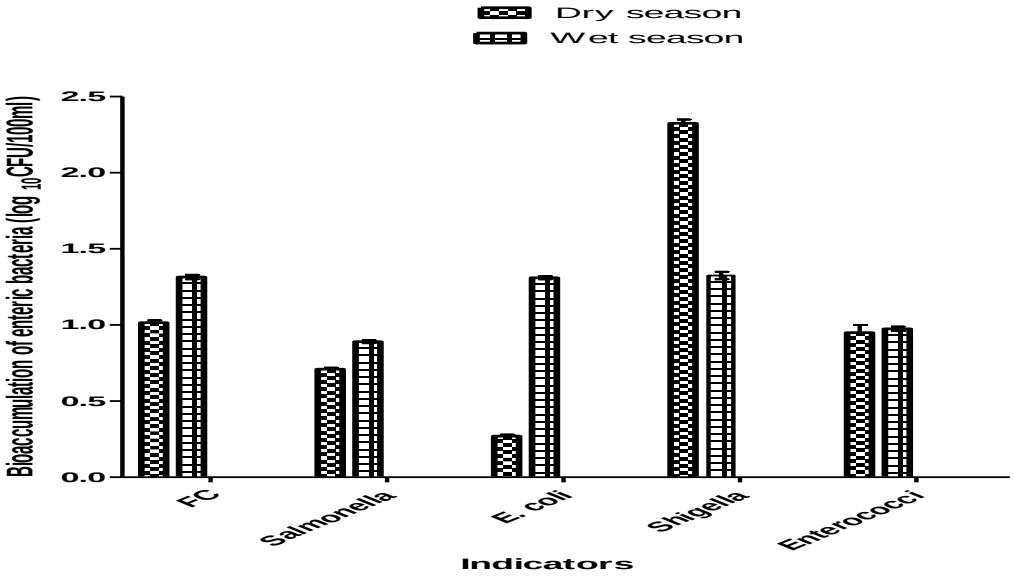
In the concrete ponds, the mean values of bioaccumulation (log_{10} CFU/100ml) of enterococci in catfish samples in dry and wet periods were 0.5 and 0.93 respectively while the mean values of bioaccumulation of faecal coli-

forms in catfish samples in dry and wet periods were 0.85 and 1.06 respectively. The mean values of bioaccumulation (log_{10} CFU/100ml) of *E. coli* in catfish samples in dry and wet periods were 0.21 and 1.07 respectively. The mean values of bioaccumulation (log_{10} CFU/100ml) of *Salmonella* in catfish samples in dry and wet periods were 0.52 and 0.80 respectively while the mean values of bioaccumulation of *Shigella* in catfish samples in dry and wet periods were 1.77 and 1.07 respectively (Fig 10). The relationship of seasonality and bioaccumulation showed that seasonality played major roles in the rate of bioaccumulation of enteric bacteria in catfish samples from the earthen and concrete ponds.



FC- Faecal coliforms

Fig 9: The rate of bioaccumulation of enteric bacteria in the catfish samples from the earthen pond during wet and dry periods



FC- Faecal coliforms

Fig 10: The rate of bioaccumulation of enteric bacteria in the catfish samples from the concrete pond during wet and dry periods

Discussion:

The assessment of faecal contamination in selected concrete and earthen ponds stocked with *Clarias gariepinus* in a fish farm was investigated in this study. The load of enteric bacteria in water and the catfish samples were in agreement with Njoku (14) who observed that the load of heterotrophic bacteria in pond water fluctuated between 0.01 and 8.7×10^5 CFU/ml, which was consistent with the load of enteric bacteria in water and catfish samples. The earthen pond had a higher repertoire of faecal contamination than the concrete pond, which could be due to faecal coliforms in generally consisting part of *Enterobacteriaceae* group that thrive well at temperature ranges of between 26ºC and 29ºC observed in earthen and concrete ponds respectively in this study.

Increased rainfall, as well as subsequent contamination from both direct and diffuse sources, may have contributed to the high amounts of faecal coliforms found in the earthen pond (15). This is consistent with the study of Ajayi and Okoh (16), who reported that microbial load is typically higher in earthen ponds as a result of natural nutrients in soils that promote microbial development. The load of faecal coliforms in catfish samples and their growing waters from the earthen and concrete ponds was found to be high, which could be due to increased temperature (26-30°C) of the developing water and increased dissolved nutrient in the growing water following catfish feeding. This is consistent with the findings of Wyatt et al., (17) in which the increased temperature and dissolved nutrients in catfish growing waters influenced the load of faecal coliforms in the catfish samples and their growing waters. Furthermore, the higher load of faecal coliforms in catfish samples and their growing waters in the earthen pond compared to the concrete pond could be attributed to the natural habitat of the earthen pond, which provides vital nutrients and minerals that may support microbial growth better than the concrete pond. This is consistent with the findings of Njoku et al., (14), who attributed the high load of faecal coliforms in the growing water samples of the earthen ponds to the natural propensity of the environment to foster development of microbes.

The load of *Salmonella* in the catfish samples and their growing waters from the earthen and concrete pond was also high in our study. This is in line with the findings of Olalemi (15), who concluded that indigenous and non-indigenous bacterial infections could be linked to fresh fish or their environment. *Salmonella* is not a recognized typical bacterial flora of catfish, and its prevalence is usually associated to its breeding, poor hygiene measures and inappropriate handling as reported by Ajayi and Okoh (16), who also confirmed that *Salmonella* can survive transitorily in the gastrointestinal system of fish. The load of *Shigella* in catfish samples and their growing waters from the earthen and concrete pond was equally high in our study. According to Salome and Faith (17), the greater load is potentially sustained by amplified nutrient load as a result of flood and runoffs into the ponds. The load of intestinal enterococci in catfish samples in the earthen and concrete ponds that were high may be as a result of the bacteriological profile of *C. gariepinus* as reported by Omeji et al., (18) where water contaminated with faecal streptococci have the tendency to infect fish in ponds.

The distribution pattern of the faecal coliforms and *Salmonella* in catfish samples of earthen and concrete ponds may be the result of use of maggots from chicken dungs as feeds and these may serve as reservoir for these bacteria, while the deviation in the distribution pattern of enterococci, *Shigella* and *E. coli* in the catfish samples in earthen and concrete ponds may be as a result of change in varying environmental conditions. The distribution pattern of faecal indicator bacteria in earthen and concrete ponds in our study agrees with the study of Ganesh et al., (19), who reported that divergence in the distribution of heterotrophic and total aquatic bacteria within the water layers could be linked to various factors affecting the distribution pattern of bacteria in fish ponds.

In our study, the mean temperature values varied from 25ºC to 30°C, which is remarkably comparable to the findings of Ntengwe and Edema (20). The water temperature reading could have been impacted by the weather conditions during the sam pling process (21). In the earthen and concrete ponds, the pH of the growing waters was 7.00.19 and 6.950.07, respectively. These pH levels are consistent with the findings of Ehiagbonare and Ogunrinde (22), who reported that pH 6 to 9 is a basic prerequisite for improved fish production. The ideal pH range for ponds was also reported

by Stone and Thomforde (23) to be 5.5 - 10.0 in order to maintain good pond productivity and fish health. It is worth noting that the results of this study are consistent with those of Ntengwe and Edema (20), who studied the physicochemical and microbiological features of water for fish production in tiny ponds. The electrical conductivity of the pond water was influenced by climatic conditions such as rainfall that occurred during the sample period, since surface runoff that contained substantial nutrients during the wet season was probably one of the variables that raised the pond water conductivity (21). The electrical conductivity values, on the other hand, were still within the normal range for fish rearing (10–1000µS/cm) (22). Dissolved oxygen condenses as a result of boost in water temperature, respiration and organic matter breakdown by aerobic aquatic organisms (23).

Within the allowed limit of 200 meq/L, the mean alkalinity values of earthen and concrete ponds were 109 and 107 meq/L respectively (24). Alkalinity levels of 75 to 200 meq/L have been reported by Rana and Jain (25), but not less than 20 meq/L is optimum in an aquaculture pond. For catfish development and good pond productivity, Rana and Jain (25) advised total alkalinity values of at least 20 meq/L. The high mean alkalinity of pond water in our study implies that even a small amount of acid will not cause a pH change. In the growing waters of the earthen and concrete ponds, dissolved oxygen (DO) was 11.952.13 and 4.66. 29 mg/L respectively. Despite the fact that the minimum amount of dissolved oxygen for tropical fish should be 5mg/L (26), the high amounts of dissolved oxygen found in this study could be due to photosynthetic activities of primary producers that comprise an elevated bio-assortment of plants especially in the earthen pond. The mean total dissolved solids (TDS) in this study were below the standard permissible limit of 1000 mg/L (27).

The biological oxygen demand (BOD) of the growing waters in the earthen and concrete ponds respectively were 2.531. 23% and 1.530.03% mg/L. The high BOD depletes oxygen levels to dangerous levels, indicating that the water is polluted. According to Bhatnagar and Singh (28), only BOD values between 3.0-6.0 mg/L are most advantageous for normal activities of catfishes while level of 6.0-12.0 mg/L is toxic to catfishes and >12.0 mg/L could effectively cause their death through suffocation. The chemical oxygen demand (COD) was 199. 62.55 mg/L in one case and 202.53.54 mg/L in another. The average chemical oxygen demand in the ponds in the current study exceeded the standard allowable limit of 10 mg/L (29).

The negative correlation of *Salmonella* with physicochemical parameters in this study was similarly reported by Olalemi and Oluyemi (30) in their study of incidence and existence of faecal pollution markers in an earthen fish pond in Akure, Nigeria. The positive correlation between turbidity and faecal coliforms seen in this study agrees with the finding of Olalemi and Oluyemi (30). Previous studies have reported that the progressive bioaccumulation of microbes in marine animals like *C. gariepinus* is influenced by an array of ecological factors (31) as evident in our study.

Conclusion:

The findings of this study revealed high levels of faecal pollution in water and African catfish (*C. gariepinus*) from earthen and concrete ponds. Physicochemical characteristics of the pond water and seasonality influenced the rate of bioaccumulation of enteric bacteria in *C. gariepinus* raised in these earthen and concrete ponds.

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Contributions of authors:

MB deduced and analyzed the study inference, wrote the original draft and edited the manuscript; OO conducted the study and collected the samples; AO conceived the study idea and reviewed the original draft. All authors agreed to the content of the final manuscript.

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Short Communication **Open Access**

Antimicrobial susceptibility of urinary bacterial isolates of pregnant women attending antenatal clinics of selected hospitals in Ilorin, Nigeria

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Abstract:

Background: Urinary tract infection (UTI) in pregnancy is associated with significant morbidity for both mother and baby. This study was aimed at determining the urinary bacterial isolates and their susceptibility to selected antibiotics among pregnant women attending antenatal clinics of selected Hospitals in Ilorin, Nigeria.
Methodology: A total of 300 pregnant women between the ages of 15 and 44 years were selected by random sampling technique from antenatal clinics of three randomly selected healthcare facilities in Ilorin, Nigeria; Civil Service Hospital, Sobi Specialist Hospital and Okelele Primary Health Center, between July and October 2021. Clean-catch mid-stream specimens of voided urine were collected from each participant, cultured on Cysteine-Lactose-Electrolyte Deficient (CLED) and Blood agar plates, and incubated aerobically at 37°C for 24 hours. The presence of significant bacteriuria ($\geq 10^5$ CFU/ml) was determined on the culture plate using the plate count method. Antibiotic susceptibility testing to selected antibiotics was done using Kirby-Bauer disk diffusion technique. Cefoxitin (30μg) was used as surrogate to determine phenotypic methicillin resistance in staphylococcus isolates, and the methicillin resistance (*mec*A) gene was detected by conventional PCR assay.
Results: Of the 300 pregnant participants, 49 (16.3%) were symptomatic for UTI while 251 (83.7%) were asymptomatic. Significant bacteria (monomicrobial) were isolated in 44 (14.7%) pregnant women; 28 (11.2%) of 251 asymptomatic and 16 (32.6%) of 49 symptomatic women, which showed that significant bacteriuria rate was higher with symptomatic than asymptomatic women (OR=3.861, 95% CI=1.889–7.893, p=0.0005), but significant bacteriuria rate did not differ with respect to age group of the women (x^2=1.463, p=0.4811). The most common bacterial isolates were *Escherichia coli* (38.6%, 17/44) followed by coagulase negative staphylococci (CoNS) (22.5%, 9/44), *Klebsiella pneumoniae* (18.2%, 8/44), *Staphylococcus aureus* (15.9%, 7/44), *Enterococcus* sp (4.6%, 2/44) and *Pseudomonas* sp (2.3%, 1/44). Gram negative isolates showed high resistance rate of 73.1% to ampicillin and 65.4% to amoxicillin-clavulanic acid while Gram-positive isolates showed high resistant rate of 94.1% to penicillin. The Gram-positive isolates showed high susceptibility rate of 100% while the Gram-negative isolates showed moderate susceptibility of 69.2% to nitrofurantoin. Four of the 9 (44.4%) CoNS isolates were cefoxitin resistant and all the 4 (100%) carried *mec*A gene.
Conclusion: The isolation of bacterial pathogens resistant to the commonly prescribed antibiotics from pregnant women symptomatic and asymptomatic for UTI calls for early screening of all pregnant women for UTI during antenatal care service delivery.

Keywords: UTI; pregnancy; bacteria; antimicrobial susceptibility; antimicrobial resistance

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Sensibilité aux antimicrobiens des isolats bactériens urinaires de femmes enceintes fréquentant des cliniques prénatales d'hôpitaux sélectionnés à Ilorin, au Nigeria

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Résumé:

Contexte: L'infection des voies urinaires (IVU) pendant la grossesse est associée à une morbidité importante pour la mère et le bébé. Cette étude visait à déterminer les isolats bactériens urinaires et leur sensibilité aux antibiotiques sélectionnés chez les femmes enceintes fréquentant les cliniques prénatales des hôpitaux sélectionnés à Ilorin, au Nigeria.
Méthodologie: Un total de 300 femmes enceintes âgées de 15 à 44 ans ont été sélectionnées par une technique d'échantillonnage aléatoire dans les cliniques prénatales de trois établissements de santé sélectionnés au hasard à Ilorin, au Nigeria; Hôpital de la fonction publique, hôpital spécialisé de Sobi et centre de santé primaire d'Okelele, entre juillet et octobre 2021. Des échantillons d'urine mictionnelle à mi-parcours ont été prélevés sur chaque participant, cultivés sur des plaques de cystéine-lactose-électrolyte déficientes (CLED) et de gélose au sang, et incubé en aérobiose à 37^{o}C pendant 24 heures. La présence d'une bactériurie significative (≥105 UFC/ml) a été déterminée sur la plaque de culture en utilisant la méthode de comptage sur plaque. Les tests de sensibilité aux antibiotiques sélectionnés ont été effectués à l'aide de la technique de diffusion sur disque de Kirby-Bauer. La céfoxitine (30μg) a été utilisée comme substitut pour déterminer la résistance phénotypique à la méthicilline dans les isolats de staphylocoques, et le gène de résistance à la méthicilline (*mec*A) a été détecté par un test PCR conventionnel.
Résultats: Sur les 300 participantes enceintes, 49 (16,3%) étaient symptomatiques d'infections urinaires tandis que 251 (83,7%) étaient asymptomatiques. Des bactéries significatives (monomicrobiennes) ont été isolées chez 44 (14,7%) femmes enceintes; 28 (11,2%) des 251 femmes asymptomatiques et 16 (32,6%) des 49 femmes symptomatiques, ce qui a montré que le taux de bactériurie significative était plus élevé chez les femmes symptomatiques que chez les femmes asymptomatiques (OR=3,861, IC 95%=1,889–7,893, p=0,00050, mais le taux significatif de bactériurie ne différait pas selon le groupe d'âge des femmes (x^2=1,463, p=0,4811). Les isolats bactériens les plus courants étaient *Escherichia coli* (38,6%, 17/44) suivi du staphylocoque coagulase négatif (CoNS) (22,5%, 9/44), *Klebsiella pneumoniae* (18,2%, 8/44), *Staphylococcus aureus* (15,9%, 7/44), *Enterococcus* sp (4,6%, 2/44) et *Pseudomonas* sp (2,3%, 1/44). Les isolats à Gram négatif ont montré un taux de résistance élevé de 73,1% à l'ampicilline et de 65,4% à l'amoxicilline-acide clavulanique, tandis que les isolats à Gram positif ont montré un taux de résistance élevé de 94,1% à la pénicilline. Les isolats à Gram positif ont montré un taux de sensibilité élevé de 100%, tandis que Les isolats à Gram négatif ont montré une sensibilité modérée de 69,2% à la nitrofurantoïne. Quatre des 9 (44,4%) isolats de CoNS étaient cefoxitin résistants et tous les 4 (100%) portaient le gène *mec*A.
Conclusion: L'isolement des pathogènes bactériens résistants aux antibiotiques couramment prescrits chez les femmes enceintes symptomatiques et asymptomatiques pour les infections urinaires nécessite un dépistage précoce des infections urinaires chez toutes les femmes enceintes lors de la prestation des services de soins prénatals.

Mots clés: UTI; grossesse; bactéries; sensibilité aux antimicrobiens; résistance antimicrobienne

Introduction:

Urinary tract infection (UTI) is an infection caused by the growth and multiplication of microorganisms in the urinary tract. It is usually due to bacteria from the gastrointestinal tracts which ascend the urethra and multiply to cause infection (1). In contrast to men, women are more susceptible to UTI, and this is mainly due to the short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (2,3). Urinary tract infection in pregnancy is associated with significant morbidity for both mother and baby. The combination of mechanical, hormonal and physiologic changes during pregnancy contributes to significant changes in the urinary tract, which has a profound impact on the acquisition and natural history of bacteriuria during pregnancy (4).

In women, UTI account for about 25% of all infections thus being one of the most frequent clinical bacterial infections (5). Its occurrence usually starts in 6 weeks and becomes most frequent during the 22 weeks of pregnancy. Untreated UTI in pregnant women may have serious consequences like intrauterine growth restriction, preeclampsia, caesarean delivery and preterm deliveries (6,7). UTI can be either symptomatic or asymptomatic. Patients with significant bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria (8,9). Asymptomatic bacteriuria (ASB) is a condition characterized by the presence of bacteria in two consecutive clear-voided urine specimens both yielding positive cultures (≥10^5 CFU/ml) of the same uropathogen in a patient without classical symptoms (10).

Escherichia coli is the major etiological agent causing UTI, which accounts for up to 90% of cases. *Proteus mirabilis, Klebsiella* sp. *Pseudomonas aeruginosa* and *Enterobacte*r sp. are less frequent offenders (11,12) and less co-

mmonly, Enterococci, *Gardnerella vaginalis* and *Ureaplasma urealyticum* are also known agents in UTI. Gram-positive organisms are even less common in which Group B *Streptococcus*, *Staphylococcus aureus,* coagulase negative staphy lococci and *Staphylococcus haemolyticus* are recognized organisms (13).

The current management of UTI is usually empirical without the use of urine culture or susceptibility testing to guide therapy. However, as with many community-acquired infections, antimicrobial resistance among the pathogens that cause UTI is increasing and is a major health challenge in the treatment of UTI (14). There is growing concern regarding antimicrobial resistance worldwide (15). Proper investigation and prompt treatment are needed to prevent serious life-threatening conditions and morbidity due to UTI that can occur in pregnant women (16). In most developing countries including Nigeria, screening for UTI in pregnancy is not considered as an essential part of antenatal care. Therefore, this study was designed to determine the urinary bacterial profile and antibiotic susceptibility of bacterial uropathogens among pregnant women attending antenatal clinics of selected hospitals in Ilorin, Kwara State, Nigeria.

Materials and method:

Study setting and selection:

The study was conducted at three different hospitals in Ilorin, Kwara State, Nigeria. These were Civil Service Clinic, Sobi Specialist Hospital and Okelele Primary Health Center.

Ethical approval

Ethical clearance was obtained from the Ministry of Health and informed consent was obtained from eligible pregnant women.

Study population, sample size and selection:

The study population were pregnant women attending the antenatal clinics of the three selected healthcare facilities. The sample size of 300 pregnant women for the study was calculated using the Cochran formula, $n = Z^2 P (1-P)/d^2$ (17). Pregnant women within the age range of 15-44 years were selected using random sampling method between July and October 2021 until the sample size of 300 was attained.

The inclusion criteria were pregnant women symptomatic and asymptomatic for UTI at the study sites who were not on antibiotics treatment during sample collection and who also gave informed consent to participate in the study. Pregnant women who did not give informed consent and who were on antibiotic treatment during sample collection were excluded.

Data and sample collection:

A structured questionnaire was used to collect socio-demographic and clinical data from the participants. All the pregnant women were trained on the standard procedure of collecting clean catch mid-stream voided urine into sterile universal bottle. The urine samples were then transported in an ice-pack to the microbiology laboratory of the University of Ilorin Teaching Hospital for analysis.

Culture isolation and bacterial identification:

A calibrated wire loop (0.001ml) was used to inoculate urine sample on CLED and Blood agar plate and the plates were incubated at 37^{o}C for 24 hrs. Colony counts were carried out on culture plates and those with colony count of $\geq 10^5$ CFU/ml were considered significant. The method employed in the identification and characterization of isolated bacteria included examination of morphological features of the colonies on the agar plates and convectional biochemical tests (18).

Antibiotic susceptibility test:

The Kirby–Bauer disk diffusion method was used to determine the antibiotic susceptibility of the isolates using selected antibiotic discs (Oxoid, UK). A colony each was inoculated into normal saline and the inoculum was standardized by comparing with 0.5 McFarland turbidity standard that gives bacterial concentration of approximately $1.5x10^8$ colony forming units per ml (CFU/ml). About 0.1ml of the inoculum was dropped and spread on Mueller-Hinton agar plate using sterile swab stick. Six antibiotic discs per plate were placed 60^0 apart on the plate. After 24 hours incubation at 37°C, the diameter of the inhibitory zone surrounding the discs was measured.

The interpretation of isolate as susceptible or resistant was done using the interpretative standards of the Clinical and Laboratory Standards Institute (19). Antibiotics discs used were penicillin (5µg), ampicillin (10µg), amoxicillin-clavulanic acid (30µg), cefuroxime (30 µg), ceftazidime (30µg), ceftriaxone (30µg), nitrofurantoin (300µg) and clindamycin (5µg). *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as control strains. Methicillin resistance in coagulase negative staphylococcus (CoNS) isolates was determined using cefoxitin disc (as surrogate) on Mueller-Hinton agar plate and those resistant to cefoxitin were screened for *mecA* gene by conventional polymerase chain reaction.

Statistical analysis of data:

Data were presented as simple frequency distribution tables and analysed using the GraphPad software (San Diego 92130, USA). Association between categorical variables was determined using Chi-squared test with Odds ratio (OR) and 95% confidence interval (95% CI). P value less than 0.05 was considered as statistical significance.

Results:

In this study, a total of 300 pregnant women, 49 (16.3%) with symptoms and 251 (83.7%) without symptoms of UTI were investigated. The age range of the participants was 15-44 years (mean age 26.7±4.7 years). Majority 175 (58.3%) of the study participants were in the age range of 25-34 years. Among the participants, 188 (62.6%) were self-employed (Table 1). The distribution of bacteriuria based on age group of participants showed that 13 (12.7%) of 102 women in age group 15-24 years were positive for bacteriuria, 29 of 175 women (16.6%) in age group 25-34 and 2 of 23 (8.7%) in age group 35-44 years were positive for significant bacteriuria (X^2=1.463, p= 0.4811) (Table 2).

Six bacteria species were isolated from the urine samples of 44 (14.7%) of the 300 pregnant women who had significant growth (>10^5 CFU/ml) of bacteria in their urine with 28 (11.2%) isolates from 251 asymptomatic and 16 (32.7%) isolates from 49 symptomatic women (OR = 3.861, 95% CI 1.889 - 7.893, p = 0.0005). The predominantly isolated bacteria were *Escherichia coli* 17 (5.7%) followed by coagulase negative staphylococcus 9 (3.0%), *Klebsiella pneumoniae* 8 (2.7%)*, Staphylococcus aureus* 7 (2.3%), *Enterococcus* species 2 (0.7%) and *Pseudomonas* species 1 (0.3%) (Table 3).

The majority of isolated Gram-negative uropathogens showed resistance rate of 73.1% (19/26) to ampicillin and 65.4% (17/26) to amoxicillin-clavulanic acid, and resistance rate to ceftriaxone, cefotaxime and cefuroxime ranged from 30.8% (8/26) to 46.2% (12/26). However, all Gram-negative bacteria isolates showed a relatively low rates of resistance to nitrofurantoin 15.4% (4/26) and ceftazidime 26.9% (7/26) (Table 4).

Table 1: Sociodemographic and clinical characteristics of the study participants

Socio-demographic variables	Frequency (%)
Age group (years)	
15- 24	102 (34.0)
25- 34	175 (58.3)
35- 44	23 (7.7)
Educational level	
Not educated	17 (5.7)
Primary	32 (10.7)
Secondary	145 (48.3)
Post-secondary	106 (35.3)
Occupational status	
Housewives	23 (7.7)
Self employed	188 (62.6)
Student	14 (4.7)
Employed	75 (25.0)
Symptoms of UTI	
Symptomatic	49 (16.3)
Asymptomatic	251 (83.7)

The Gram-positive uropathogens showed a high level of resistance of 94.1% (16/18) to penicillin. The resistance rate to ceftriaxone and amoxicillin-clavulanic acid ranged from 29.4% (5/18) to 47.1% (8/18). On the other hand, all Gram-positive isolates showed full susceptibility (100.0%) to nitrofurantoin. Moreover, 76.5% (13/18) and 58.8% (10/18) of the Gram-positive isolates were susceptible to clindamycin and amoxicillin-clavulanic acid respectively (Table 5).

Of the 9 CoNS isolates phenotypically screened for methicillin resistance by cefoxitin disc, 4 (44.4%) were resistant and all the 4 isolates (100%) carried *mec*A gene on PCR assay (Fig 1)

Table 2: Prevalence of significant bacteriuria in relation to age group of participants

Age group (years)	No of participants	No with significant bacteriuria	x^2	p value
15 – 24	102	13 (12.7)	1.463	0.4811
25 – 34	175	29 (16.6)		
35 – 44	23	2 (8.7)		
Total	**300**	**44 (14.7)**		

Table 3: Bacterial uropathogens of urinary tract infection in pregnancy with respect to symptomatology

Bacterial isolates	Symptomatic (%) (n=49)	Asymptomatic (%) (n=251)	COR (95% CI)	p value
Gram negative (n=26)	**9 (18.4)**	**17 (6.7)**	**3.097 (1.291 -7.429)**	**0.0215***
Escherichia coli	6 (12.2)	11 (4.4)		
Klebsiella pneumoniae	2 (4.1)	6 (2.4)		
Pseudomonas sp	1 (2.0)	0		
Gram-positive (n=18)	**7 (14.3)**	**11 (4.4)**	**3.636 (1.334 – 9.914)**	**0.0154***
Staphylococcus aureus	3 (6.1)	4 (1.6)		
CoNS	2 (4.1)	7 (2.8)		
Enterococcus sp	2 (4.1)	0		
Total (n=44)	**16 (32.7)**	**28 (11.2)**	**3.861 (1.889 – 7.893)**	**0.0005***

CoNS=coagulase negative staphylococcus; COR=crude ood ratio; CI=confidence interval n=number

Table 4: *Invitro* antibiotic susceptibility of Gram-negative urinary bacterial isolates from pregnant women to selected antibiotics

Isolate/Antibiotic		AMP	AUG	CXM	CRO	CTX	CAZ	NF
Escherichia coli (n=17)	S	1 (5.9)	3 (17.6)	3 (17.6)	9 (52.9)	6 (35.3)	8 (47.1)	13 (76.5)
	I	1 (5.9)	2 (11.8)	4 (23.5)	2 (11.8)	4 (23.5)	4 (23.5)	1 (5.9)
	R	15 (88.2)	12 (70.6)	10 (58.8)	6 (35.3)	7 (41.2)	5 (29.4)	3 (17.6)
Klebsiella pneumoniae	S	3 (37.5)	1 (12.5)	4 (50.0)	5 (62.5)	3 (37.5)	5 (62.5)	5 (62.5)
(n=8)	I	2 (25.0)	3 (37.5)	3 (37.5)	2 (25.0)	2 (25.0)	1 (12.5)	3 (37.5)
	R	3 (37.5)	4 (50.0)	1 (12.5)	1 (12.5)	3 (37.5)	2 (25.0)	0
Pseudomonas spp (n=1)	S	0	0	0	0	1 (100)	1 (100)	0
	R	1 (100)	1 (100)	1 (100)	1 (100)	0	0	1 (100)
Total (n=26)	S	4 (15.4)	4 (15.4)	7 (26.9)	14 (53.8)	10 (38.5)	14 (53.8)	18 (69.2)
	I	5 (19.2)	5 (19.2)	7 (26.9)	4 (15.4)	6 (23.1)	5 (19.2)	4 (15.4)
	R	19 (73.1)	17 (65.4)	12 (46.2)	8 (30.8)	10 (38.5)	7 (26.9)	4 (15.4)

AMP= Ampicillin; AUG= Amoxicillin-clavulanic acid; CXM = Cefuroxime; CRO = Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; NF= Nitrofurantoin, R= Resistant; I= Intermediate; S= Sensitive.

Table 4: *Invitro* antibiotic susceptibility of Gram-positive urinary bacterial isolates from pregnant women to selected antibiotics

Isolate/Antibiotic		PEN	AUG	CRO	CL	NF
Staphylococcus aureus (n=7)	S	0	4 (66.7)	1 (16.7)	4 (66.7)	6 (100)
	I	0	0	0	1	0
	R	6 (100)	2 (33.3)	5 (83.3)	1 (16.7)	0
Coagulase negative staphylococcus (n=8)	S	0	4 (44.4)	4 (44.4)	7 (77.8)	9 (100)
	I	0	2 (22.2)	2 (22.2)	1 (11.1)	0
	R	9 (100)	3 (33.3)	3 (33.3)	1 (11.1)	0
Enterococcus spp (n=2)	S	1 (50.0)	2 (100)	2 (100)	2 (100)	2 (100)
	R	1 (50.0)	0	0	0	0
Total (n=17)	S	1 (5.9)	10 (58.8)	7 (41.1)	13 (76.5)	17 (100)
	I	0	2 (11.8)	2 (11.8)	2 (11.8)	0
	R	16 (94.1)	5 (29.4)	8 (47.1)	2 (11.8)	0

CoNS=coagulase negative staphylococcus; PEN=penicillin; AUG=amoxicillin-clavulanic acid; CRO= ceftriaxone; CL=clindamycin; NF=nitrofurantoin; R= resistant, I= intermediate, S=sensitive.

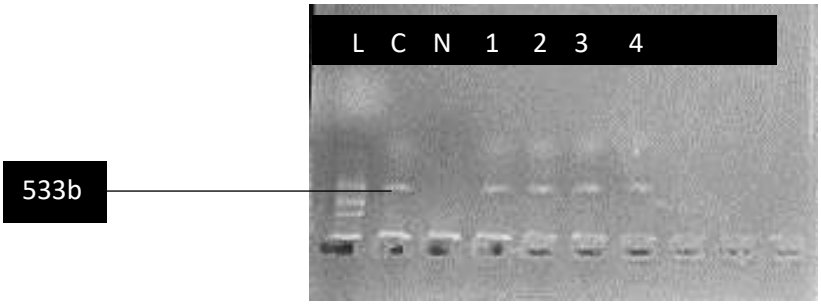

Fig 1: Gel electrophoresis showing amplified *mec*A gene product (533bp) in four CoNS isolates

Discussion:

The prevalence of UTI in this study was 14.5%, which is lower compared to previous studies in Nigeria such as 27.9% reported in Ilorin (20), 43.3% in Abuja (21), 26% in Kaduna (22), 61.5% in Aba (23) and 15.8% in Kano (24). However, the prevalence is higher than 10.3% reported in Plateau State (25) and 10.6% in Enugu (26). Prevalent rates of 6.1% to 10.9% have been reported in Iran (27) while Obirikorang et al., (28) reported 7.3% prevalence in Ghana. These wide differences in prevalence rates may be due to differences in the study population and geographic locations. The highest prevalence of the bacteriuria was seen among the pregnant women in age group 25-34 years (16.6%), which is similar to the finding of Banda et al., (29) who reported highest rate of significant bacteriuria in the age group of 24-28 years, but contrast the studies of Turpin et al., (30) and Amadi et al., (31), who reported higher prevalence rate in pregnant women aged 35 to 39 years. In our study, age group 25-34 years with the highest prevalence of significant bacteriuria is sexually active age group and therefore are at higher risk of developing urinary tract infection.

In this study, a total of 44 isolates were obtained from the 44 pregnant women with significant bacteriuria, with only one bacterial specie isolated from each woman indicating the mono-microbial nature of significant bacteriuria and UTI in the study population. The most frequent urinary bacterial isolate in symptomatic and asymptomatic pregnant women was *E. coli*, responsible for 38.6% (17/44) of the isolates and 5.7% (17/300) of the women. This was followed by CoNS (22.7%), *K. pneumoniae* (18.2%), *S. aureus* (13.6%) and *P. aeruginosa* (2.3%). This finding is similar to other studies which reported that Gram-negative bacteria, particularly *E. coli,* as the commonest pathogens isolated in patients with UTI (32). In a similar study by Kelkewa et al., (33), the commonest isolates were also *E. coli* followed by *S. aureus.* Similar pattern was also reported by Beleta et al., (34) but this was contrary to this present study in which CoNS was the next frequently isolated pathogen (22.7%). The frequency of 18.2% for *K. pneumoniae* in our study shows that *Klebsiella* species are gaining more prominence as aetiological agents of bacteriuria and UTI than previously reported *(*7).

The majority of isolated Gram-negative uropathogens in this study showed high resistance to ampicillin and amoxicillin-clavulanic acid (augmentin) and moderate resistance to ceftriaxone, cefotaxime and cefuroxime. This is similar to the study of Balakrishnan et al., (35) who reported that Gram-negative isolates were highly resistant to augmentin and ceftriaxone. However, all Gram-negative bacteria isolate were highly susceptible to nitrofurantoin, consistent with the study of Yeva et al., (36) where most of the isolates were sensitive to nitrofurantoin. Also, the Gram-positive isolates were fully susceptible (100.0%) to nitrofurantoin, similar to the study of Mona et al., (37), which reported that Gram-positive bacteria were highly susceptible to nitrofurantoin. However, the Gram-positive uropathogens showed a high level of resistance for penicillin, as similarly reported by Rosana et al., (38). Indiscriminate use of antibiotics, irrational prescribing and unregulated use of antibiotics in animal husbandry may be responsible for this increasing antibiotic resistance (39).

The CoNS isolates were the most frequent Gram-positive bacteria in this study which formed 9 (20.5%) out of the total 44 bacterial isolates, 4 (44.4%) of which were resistant to cefoxitin, a surrogate marker of methicillin resistance. This rate is lower than the study of May et al., (40) who reported 66.7% of their 12 CoNS to be resistant to cefoxitin. All the 4 (100) cefoxitin resistant CoNS carried *mec*A

gene on PCR assay, similar to the study of Manon et al., (41) in which 93% of cefoxitin-resistant CoNS carried *mec*A gene, but contrast the findings of Bale et al., (42) in which only 75% of cefoxitin resistant CoNS carried *mec*A gene. This variation could be as a result of differences in the species and strains of CoNS as well as sample size of isolates tested in the different studies.

Conclusion:

This study confirmed that after *E. coli*, CoNS were the most common bacterial isolates involved in urinary tract infection in pregnant women in the study population. The isolation of bacterial pathogens from both symptomatic and asymptomatic pregnant women that are resistant to the commonly prescribed antibiotics and the high presence of *mec*A genes in the CoNS isolates calls for early screening of all pregnant women for UTI. Early diagnosis and treatment of UTI during pregnancy can ensure the safety of the mother and fetus and also prevent complications during delivery.

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Contribution of authors:

RT carried out the research; MR and AS supervised the microbiological analysis; and MI supervised the molecular analysis.

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Conflict of interest:

No conflict of interest is declared.

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Letter to the Editor **Open Access**

African Traditional Medicine (ATM) Day 2022

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Journée de la Médecine Traditionnelle Africaine (ATM) 2022

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Dear Editor,

Since the year 2000, 31st of August of every year has been set aside by the World Health Organization (WHO) to celebrate the contributions of African Traditional Medicine (ATM). The 2022 commemoration highlights the challenges against the development of ATM in Nigeria and how they can be overcome; how the country can maximize it to boost foreign exchange earnings and health care; and what role ATM can play in addressing the rise in viral and infectious diseases such as monkeypox, COVID-19, Marburg virus, Langya Henipavirus, Polio and others. This letter addresses the above issues raised by Nigerian Office of the WHO.

According to the WHO, Traditional Medicine refers to the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health and in the diagnosis, improvement or treatment, and prevention of physical and mental illness (1). Up to 80% of the population depends on ATM either primarily or as an adjunct to orthodox medicine for their health needs. The main issues with ATM practice in Nigeria are enumerated as follows;

What have been the challenges towards the development of traditional medicine?

The followings highlight some of the challenges of ATM; (i) many traditional medicine practitioners use both incantations and herbal medicines for treatment of patients. This practice makes traditional medicine appears mysterious, unscientific and therefore unacceptable to a large proportion of the populations (2); (ii) many herbal drugs are prepared with hot alcoholic drinks that can damage the kidneys and the liver, especially in children (3); (iii) inadequate knowledge of the mode of action, excretion and dosages of the herbal drugs; (iv) unhygienic environments of the herbal medical practices; (v) lack of approval by health regulatory bodies; (vi) inadequate training of the herbal practitioners; and (vii) lack of publications of herbal drugs in high impact peer-reviewed journals.

How can Nigeria overcome these challenges?

The following suggestions are enumerated; (i) demystifying the use of native medicines, with separation of incantations from the use of medicines; (ii) extraction of active components of the leaves, fruits, seeds, backs or roots of the herbal plants; (iii) conducting pharmacodynamics and pharmacokinetic studies on the extracts; and attaining appropriate dosing of these medicines; (iv) conducting toxicological tests of the drugs on laboratory animals. In early August 2022, the National Agency for Food Drug Administration and Control (NAFDAC) withdrew approval for five previously approved alcoholic bitters because they cause kidney damage; (v) conducting clinical trials for efficacy and safety of the drugs; (vi) publications of the research outcomes in peer-reviewed scientific journals; (vii) inclusion of ATM in national health policies and national health strategic plans; (viii) encou-

raging healthy collaboration between ATM and orthodox medicine practitioners; (ix) increase funding for researches on medicinal herbs; (x) establishment of regulatory body for ATM and its practitioners, code of ethics for ATM practitioners, setting up national research institutes dedicated to ATM, registration and local production of traditional medicine products; (xi) cultivation and growth of medicinal plants; (xii) institutionalised training and re-training programmes for practitioners of ATM; (xiii) national plan for integrating ATM into the country's Primary Health Care (PHC) system; (xiv) incorporating Traditional Birth Attendance(TBA), as currently being done in Lagos State health care system through collaboration with orthodox medicine, training and re-training of TBA practitioners on when to refer a woman in labour to the near-by hospital. This is yielding positive results and should be emulated by other States of the Federation (4). This form of collaboration should also be extended to other units of medical care, for instance, the Traditional Bone Setter (TBS) practitioners should be seen by the Orthopaedic surgeons as partners in progress and not as antagonists or nonentities.

How can the country maximize ATM to boost foreign exchange earnings and health care?

The Government of Nigeria is making deliberate efforts towards maximizing traditional medicine to boost healthcare and foreign earnings; (i) The Government, through the Ministry of Health, recently held a conference of Traditional, Complementary and Alternative Medicine (TCAM) in June 2022. The Minister of Health, Dr Osagie Ehanire, stated at the conference that plans were in the pipeline to ensure that traditional medicine practice is legally absorbed into the PHC of the country (5). The main objective of the conference was to encourage the growth and utilization of the over 10,000 species of medicinal plants that Nigeria is endowed with as potential sources of raw materials for the Pharmaceutical, Food and Cosmetic industries within and outside the country. This will create direct and indirect job opportunities for Nigerians. This can be used to boost foreign exchange earnings through short, medium, and long-term value chain and by attracting agricultural business loans and Bank of Industries (BOI) manufacturing loans. This will increase Nigerian benefit from the global herbal medicine market largely dominated by China, United States, Germany, India, and Thailand, and is projected to reach seven trillion USD by 2050 (5).

What role can ATM play in addressing the rise in viral and infectious diseases such as Monkeypox, COVID-19, Marburg virus, Langya Henipavirus, Poliovirus, and others?

The following are some of the promising plants; (i) *Securidaca lonipedunculata* (violet tree), also called *Sanya* (Hausa) or *Ipeta* (Yoruba) is an anecdotal plant that is believed to treat all ailments. It is a well-respected plant among the Hausa tribe that they refer to it as *Umar magunguna* meaning, "mother of medicines, the yellow roots of which is normally chewed in the mouth; (ii) *Enantia chlorantia*, also called *Awopa*, *Osopupa*, *Dokita Igbo* in Yoruba is another legendary plant. Others are wild grapes (for asthma), neem tree (for malaria), and guava leaves tea (as an immune booster) (6,7). In a recent study by Abubakar et al., (8), a number of diseases such as hepatitis, poliomyelitis, monkeypox, smallpox, yellow fever, Lassa fever, meningitis, and COVID-19 were reported to have been treated with some selected medicinal plants in some northern Nigerian States (Katsina, Kebbi, Kwara and Sokoto), with moderate success.

Conclusion:

The future of traditional medicine in Nigeria is very bright if all stakeholders play their due roles for the growth and development of the sector.

Contributions of authors:

BA designed the project, searched the literature and reviewed the manuscript. JI also searched online literature and wrote the first draft. All authors agreed on the final manuscript.

Conflicts of interest:

Authors declared no conflict of interest

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