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TABLE OF CONTENTS

Review Article   **Open Access**

The role of infections in the pathogenesis of bleeding among patients with haemophilia-A: A primer for haemophilia caregivers in the tropics

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Abstract:

Background: Haemophiliacs are often transfusion-dependent, and are at risk of HIV and non-HIV immuno-suppression, making them vulnerable to transfusion-transmissible infections (TTIs) and non-TTIs, many of which can cause infection-associated bleeding (IAB) even in non-haemophilic individuals. Haemophiliacs are particularly susceptible to IAB due to vicious interaction between pre-existing 'inherited' FVIII deficiency and infection-induced 'acquired' pro-haemorrhagic abnormalities. IAB in haemophiliacs manifests as undue musculoskeletal and/or mucocutaneous haemorrhages. It is thus important for haemophilia caregivers in general (and in the tropics in particular) to have thorough understanding of IAB. Clinico-pathological perspectives of IAB in haemophilia are fragmented, and not comprehensively appraised in previous literature. This review presents updated, compre-hensive but concise overview of pathogenesis, trigger mechanisms, clinical implications, therapy and prevention of IAB in haemophiliacs as accrued from literature.
Methodology: Online databases such as PubMed, Medline, Google Scholar and others were interrogated using the search terms; 'haemophilia-A', 'viral, bacterial and parasitic infections', 'bleeding', 'mucocutaneous', 'thrombo-cytopenia', 'ecchymosis', 'purpura', 'haematuria', 'melena', 'haematemesis', and 'haemoptysis' in various combina-tions.
Results: Pathogenesis of IAB in haemophilia include mucosal ulcerations, acquired coagulopathy, and/or portal hypertension. As long as the causative infections are untreated, IAB is often persistent or recurrent, predisposing patients to absenteeism from school/work, iron deficiency, excessive exposure to blood products, high risk of acquiring additional TTIs and increased risk of developing inhibitors to FVIII. Haemophilia caregivers should investigate stool, urine, sputum, blood and/or radiographs of all cases of persistent or recurrent bleeding, especially if bleeding is unabated by blood products transfusion alone, and more-so in patients presenting with constitutional and/or systemic indicators of infections such as pyrexia, asthenia, dysuria, cough, diarrhoea, jaundice, or history of passage of worms in the stool. Transfusion of blood products alone would not suffice for IAB, and transfusions of FVIII containing products without concurrent anti-infection chemotherapy may even promote the development of FVIII inhibitors since active infections and inflammations are important risk factors for inhibitor development in haemophiliacs.
Conclusion: It is therapeutically essential to combine transfusion therapy with anti-infective chemotherapy in order to achieve prompt and sustained stoppage of IAB. Haemophilia caregivers should also counsel patients on hygiene, barrier protection against vectors, and vaccination protocols.

Keywords: Haemophilic bleeding; bacteria, viral, parasitic, infections; pathogenesis

Le rôle des infections dans la pathogenèse des hémorragies chez les patients atteints d'hémophilie A: une introduction pour les soignants hémophiles sous les tropiques

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Résumé:

Contexte: Les hémophiles sont souvent dépendants des transfusions et courent un risque d'immunosuppression liée au VIH et non liée au VIH, ce qui les rend vulnérables aux infections transmissibles par transfusion (ITT) et non-ITT, dont beaucoup peuvent causer des saignements associés à l'infection (IAB) même chez les personnes non hémophiles. Les hémophiles sont particulièrement sensibles à l'IAB en raison de l'interaction vicieuse entre le déficit préexistant en FVIII «héréditaire» et les anomalies pro-hémorragiques «acquises» induites par l'infection. L'IAB chez les hémophiles se manifeste par des hémorragies musculo-squelettiques et/ou cutanéo-muqueuses excessives. Il est donc important pour les soignants hémophiles en général (et sous les tropiques en particulier) d'avoir une compréhension approfondie de l'IAB. Les perspectives clinico-pathologiques de l'IAB dans l'hémophilie sont fragmentées et ne sont pas évaluées de manière exhaustive dans la littérature précédente. Cette revue présente un aperçu actualisé, complet mais concis de la pathogenèse, des mécanismes déclencheurs, des implications cliniques, du traitement et de la prévention de l'IAB chez les hémophiles, tels qu'ils ressortent de la littérature.
Méthodologie: Des bases de données en ligne telles que PubMed, Medline, Google Scholar et d'autres ont été interrogées à l'aide des termes de recherche; «hémophilie A», «infections virales, bactériennes et parasitaires», «hémorragies», «mucocutanées», «thrombocytopénie», «ecchymoses», «purpura», «hématurie», «méléna», «hématémèse», et «hémoptysie» dans diverses combinaisons.
Résultats: La pathogenèse de l'IAB dans l'hémophilie comprend les ulcérations muqueuses, la coagulopathie acquise et/ou l'hypertension portale. Tant que les infections causales ne sont pas traitées, l'IAB est souvent persistante ou récurrente, prédisposant les patients à l'absentéisme scolaire/au travail, à une carence en fer, à une exposition excessive aux produits sanguins, à un risque élevé de contracter des ITT supplémentaires et à un risque accru de développer des inhibiteurs du FVIII. Les soignants hémophiles doivent examiner les selles, les urines, les expectorations, le sang et/ou les radiographies de tous les cas de saignement persistant ou récurrent, en particulier si le saignement n'est pas atténué par la transfusion de produits sanguins seuls, et plus encore chez les patients présentant des indicateurs constitutionnels et/ou systémiques de infections telles que pyrexie, asthénie, dysurie, toux, diarrhée, ictère ou antécédents de passage de vers dans les selles. La transfusion de produits sanguins seuls ne suffirait pas pour l'IAB, et les transfusions de produits contenant du FVIII sans chimiothérapie anti-infectieuse concomitante peuvent même favoriser le développement d'inhibiteurs du FVIII puisque les infections actives et les inflammations sont des facteurs de risque importants pour le développement d'inhibiteurs chez les hémophiles.
Conclusion: Il est thérapeutiquement essentiel de combiner la thérapie transfusionnelle avec la chimiothérapie anti-infectieuse afin d'obtenir un arrêt rapide et durable de l'IAB. Les soignants hémophiles doivent également conseiller les patients sur l'hygiène, la barrière de protection contre les vecteurs et les protocoles de vaccination.

Mots clés: Saignement hémophilique; infections bactériennes, virales, parasitaires; pathogénèse

Introduction:

Haemophilia-A is an X-linked recessive disorder of blood coagulation characterized by lifelong bleeding diathesis due to deficiency of FVIII in the intrinsic arm of secondary haemostasis (1,2). Deficiency of FVIII leads to inadequate formation of tenase, sub-optimal generation of thrombin, insufficient deposition of fibrin and defective clot formation (1,2). The clinical severity of haemophilia is principally determined by the residual levels of FVIII, and is thus categorized as severe (FVIII level <1%), moderate (FVIII level 1-5%) or mild (FVIII level 6-40%) (1,2). There is a clear correlation between clinical severity of haemophilia and the frequency and spontaneity of bleeding episodes. Severe haemophilia is typically associated with more frequent bleeding episodes, which often occur spontaneously (1,2). Non-severe haemophilia is generally ass-ociated with less frequent bleeding episodes, which are usually triggered by obvious trauma (1,2). No organ or tissue is exempted from haemophilic bleeding (3). However, the pattern, frequency and spatial distribution of bleeding sites in haemophiliacs are significantly determined by the extent of extrinsic pathway inhibition and/or the level of tissue factor activity in any particular tissue as exemplified in four tissues (joint, skeletal muscle, brain and lungs) (3). The most classical example is the 'joint' within which the chondrocytes and synovial cells produce tissue factor pathway inhibitor, which attenuates intra-articular activity of extrinsic pathway and predisposes to recurrent haemarthrosis and chronic arthropathy (4). Another example is the 'skeletal muscle', which is only modestly endowed with procoagulant tissue factor activity (5) thus predisposing to recurrent intra-muscular bleeding and contractures (6). In contradistinction, the

'brain' has the highest concentration of tissue factor activity (5) and is thus associated with less frequent bleeding episodes (3). In similarity with the brain, the lungs have high concentration of tissue factor (5), thus spontaneous pulmonary haemorrhage and haemoptysis are rare manifestations of haemophilia (7). For these aforementioned reasons, musculoskeletal (joints and muscles) bleeding and complications are particularly common and virtually pathognomonic of haemophilia (3,6, 8). Mucocutaneous bleedings also occur in haemophilia, but at much lower relative frequencies because primary haemostasis is well preserved in haemophilia (3,6,8). This natural pattern of bleeding clinically distinguishes haemophilia from von Willebrand disease, in which primary haemostasis is severely impaired and mucocutaneous bleeding (e. g., haematuria, melena, haematemesis, epistaxis, gum bleed, ecchymosis) occurs at relatively higher frequency than musculoskeletal bleeding (8).

We thus reckon that haemophilic bleeding can occur spontaneously or be triggered by a myriad of causes, which include accidental trauma, surgical procedures, peptic ulcers, gastrointestinal irritants such as iron pills or medications that have dual effects of gastrointestinal irritation and platelet function inhibition such as non-steroidal anti-inflammatory analgesics (3,9,10). Moreover, infections are important triggers of bleeding in haemophilia, and haemophiliacs are particularly susceptible to infections for two important reasons. First haemophiliacs are usually managed by chronic transfusion of blood products (fresh whole blood, fresh plasma, cryoprecipitate, FVIII concentrate) that are known to cause undesirable immune modulation (11), which culminates in transfusion-associated immune suppression (TAIS) even in the absence of HIV infections (12). Second, chronic transfusion of blood products predisposes haemophiliacs to acquisition of transfusion transmissible infections (TTIs) including HIV (13), which may progress to AIDS and aggravate any pre-existing TAIS (14). Consequently, haemophiliacs are at high risk of acquiring both TTIs and non-TTIs, many of which are potential triggers of infection-associated bleeding (IAB) even among haemostatically normal (non-haemophilic) persons. The risk of IAB would be especially high among haemophiliacs in Africa and other tropical regions, which carry the heaviest burden of infectious diseases (15). Moreover, haemophiliacs would be particularly susceptible to IAB due to a potentially vicious interplay between pre-existing 'inherited' FVIII deficiency and infection-induced 'acquired' pro-haemorrhagic abnormalities. IAB in haemophiliacs manifests as undue musculoskeletal and/or mucocutaneous haemorrhages. There is therefore a need for thorough appraisal of IAB in haemophilia.

To the best of our knowledge based on literature search, the clinico-pathological perspectives of IAB in haemophilia are fragmented, and have neither been holistically nor comprehensively appraised in the literature. Nonetheless, IAB in haemophilia is of clinical significance for five reasons. First, IAB tends to be persistent or at best recurrent as long as the infection remains active and untreated, thereby increasing the frequency of hospital visits, which would lead to high rates of school absenteeism (in children) and work absenteeism (in adults); absenteeism is inimical to the educational and economic development of haemophiliacs (16,17). Second, the persistent and/or recurrent nature of IAB invariably increases patients' transfusion requirement, which is undesirable, particularly in the tropics where availability of blood products is low because donor inertia is high (18), female gender participation in blood donation is poor (19), voluntary donors are scarce (20), and potent antihaemophilic blood products (such as cryoprecipitate and FVIII concentrates) are often out-of-stock or unaffordable (21,22,23). Third, persistent IAB would worsen the pre-existing high prevalence of iron deficiency, which is quite common among tropical haemophiliacs due to the combined effects of poverty, malnutrition and inadequate management of bleeding episodes (24). Fourth, IAB episodes cannot be effectively controlled by transfusion of blood products alone; optimal management of IAB requires detection of causative infection, followed by synchronized application of blood products and anti-infection chemotherapy. And fifth, infusions of FVIII containing blood products without concomitant anti-infection chemo therapy may promote the development of FVIII inhibitors since active infections and inflammations are important risk factors for inhibitor development in haemophilia (25). The aforementioned five reasons underscore the need for haemophilia caregivers in general (and in the tropics in particular) to have thorough understanding of the destabilizing effects of infections on the precarious thrombo-haemorrhagic balance of haemophilia patients.

It is thus pertinent to rekindle the awareness and clinical index of suspicion of haemophilia caregivers in order to ensure that pro-haemorrhagic infections in haemophiliacs are quickly diagnosed and promptly treated. Hence, the aim of this review is to present an

updated and comprehensive but concise overview of the pathogenesis, trigger mechanisms, clinical implications, therapy and prevention of IAB among haemophiliacs as accrued from the literature.

Methodology and Results:

Literature search was conducted using relevant search terms; 'haemophilia-A', 'viral, bacterial, and parasitic infections', 'bleeding', 'mucocutaneous', 'thrombocytopenia', 'ecchymosis', 'purpura', 'haematuria', 'melena', 'haematemesis', and 'haemoptysis' in various combinations on online databases such as PubMed, Medline, Google Scholar, and others. A total of 114 publications were used for the review and this included 110 peer-reviewed journal articles, 3 technical reports of the World Health Organization (WHO), and 1 edited text book. The summary result of the literature search is outlined in Table 1

Table 1: Pathogenesis, mechanisms, clinical implications, therapy and prevention of infection associated bleeding in haemophiliacs

Categories of infection	Types of infection	Potential trigger mechanisms for bleeding	Clinical implications	Therapeutic strategy in addition to FVIII therapy	Preventive strategy
Transfusion Transmissible Viruses	HIV	HIV-associated ITP	Increased risk of MCC bleedings	Anti-viral therapy; immune modulation therapy for ITP	Improve local blood banking practices
	Hepatitis B virus	Hepatocytopathy; multi-factor deficiency; cirrhosis; portal hypertension	Increased risk of MSK and MCC bleedings	Anti-viral therapy; multi-factor concentrates; portal hypertension therapy	Improve local blood banking practices; vaccination
	Hepatitis C virus	Hepatocytopathy; multi-factor deficiency; cirrhosis; portal hypertension	Increased risk of MSK and MCC bleedings	Anti-viral therapy; multi-factor concentrates; portal hypertension therapy	Improve local blood banking practices
	Cytomegalovirus	GIT mucosal ulceration; CMV-associated ITP	Increased risk of MCC bleedings	Anti-viral therapy; immune modulation therapy for ITP	Improve local blood banking practices
Haemorrhagic Fever Viruses	Dengue Virus	Thrombocytopenia; hypofibrinogenemia	Increased risk of MCC bleedings	Platelet concentrate transfusion; fresh plasma transfusion	Nets and barrier protection; environmental vector control; vaccination
Bacteria	*Helicobacter pylori*	GIT mucosal ulceration; H pylori-associated ITP	Increased risk of gastric and other MCC bleedings	Antibiotics; anti-ulcer therapy; immune modulation therapy for ITP	Improve personal and environmental sanitation
	Mycobacterium tuberculosis	Respiratory mucosal ulceration; TB-associated ITP	Increased risk of haemoptysis and other MCC bleedings	Anti-tuberculosis chemotherapy; immune modulation therapy for ITP	Improve nutrition, personal and environmental sanitation; vaccination
Parasites	Intestinal helminths	GIT mucosal ulceration; secretion of anticoagulants by parasites	Increased risk of GIT bleedings	Anti-helminthic chemotherapy	Improve personal and environmental sanitation
	Schistosoma haematobium	Bladder mucosal ulceration; secretion of anticoagulants by parasites	Increased risk of haematuria	Anti-schistosomal chemotherapy	Improve personal and environmental sanitation
	Plasmodium species	Thrombocytopenia	Increased risk of MCC bleedings	Anti-malarial chemotherapy; platelet concentrate transfusion	Nets and barrier protection; environmental vector control; vaccination

ITP=Immune thrombocytopenic purpura; GIT=Gastrointestinal tract; MCC=Mucocutaneous; MSK=Musculoskeletal

Infections and pathogenesis of haemophilic bleeding

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Discussion:

Transfusion transmissible viral infections (TTVIs)

Viruses that are transmissible by blood transfusion possess a number of characteristics, which include having long incubation period and/or clinical latency with ability to cause sub-clinical disease and/or exist in a carrier state (26). Moreover, viruses must have significant blood phases as part of their cycles and they must remain stable and retain their infectivity during blood storage at temperatures of about 4°C for several days (26). Most transfusion transmissible viruses are either exclusively plasma-borne (extra-cellular) such as hepatitis B and C viruses that are readily transmitted by transfusion of infected plasma (and cellular products due to residual plasma) or are virtually cell associated (intra-cellular) such as cytomegalovirus (CMV) and readily transmitted by infected cellular blood products such as leucocytes and platelets concentrates but rarely transmitted by cell-free plasma (27-29). However, HIV is both plasma borne and cell-associated since it has special tropism for monocytes, T-helper lymphocytes and other CD4 receptor bearing cells (30). Therefore, HIV has significant cellular and plasma reservoirs throughout the course of infection, and is readily transmissible by both cellular concentrates and plasma derivatives (30)

Although haemophiliacs are at high risk of acquiring any transfusion transmissible viral infections (TTVIs), the aforementioned four TTVIs (HIV, hepatitis B and C, and CMV) constitute particular danger to the haemophiliacs because they are pro-haemorrhagic and have the capability to destabilize thrombo-haemorrhagic equilibrium and aggravate pre-existing haemophilic bleeding diathesis due to FVIII deficiency as described below.

HIV, HBV and HCV infections in haemophilia

These three TTVIs have comparable epidemiology in terms of their modes of spread including via sexual intercourse, blood-to-blood contacts such as unsterilized invasive procedures (e.g., injections, trado-cultural skin incisions, unhygienic surgical practices) and transfusion of inadequately screened or unscreened blood and blood products. On the one hand, sexual contact and unsterilized blood-to-blood contact may be largely responsible for the relatively high prevalence of these infections among tropical populations and apparently healthy blood donors (15). On the other hand, transfusion of inadequately screened or unscreened blood and blood products are largely responsible for high prevalence of these infections among transfusion dependent patients (including haemophiliacs) in the tropical and developing countries (13). The risks of acquiring these infections in developed countries have been greatly minimized as a result of modernization of blood safety protocols with efficient donor screening procedures, effective viral inactivation techniques and production of recombinant blood products (31). However, infection risks are particularly high in developing countries and the tropics where the prevalence of blood born infections is high among donor populations, donor screening procedures are inadequate, viral inactivation techniques are virtually absent, and recombinant blood products are unavailable (32,33). Consequently, the prevalence of TTVIs among multi-transfused haemophiliacs was reported to be as high as 24.7% in Nigeria in West Africa (34), 17.3-47.5% in Egypt, North Africa (35), while a staggering prevalence of more than 50% was previously reported from Pakistan in Asia (36). The acquisition of TTVIs has adverse clinical implications within the context of a pre-existing bleeding disorder such as haemophilia.

The hepatitis viruses (HBV and HCV) often cause chronic liver diseases (CLD) resulting in significant damage to hepatocytes, which are the main producers of all coagulation factors with the exception of FVIII and von Willebrand factor (vWF) (37). Therefore, acquisition of CLD essentially transforms haemophilia from a 'single-factor' to a 'multi-factor' deficiency disorder, which would not respond optimally to FVIII therapy alone. Hence, in addition to FVIII concentrate, haemophiliacs with liver disease may require supplementary use of blood products that contain multiple clotting factors such as fresh plasma and/or cryoprecipitate (38), anti-viral chemotherapy and immune modulators such as α-interferon (39). Moreover, CLD can cause portal hypertension, which increases hydrostatic pressure and dilatation of the abdominal veins leading to increased risk of gastrointestinal bleeding (40). In addition, viral CLD may transform into hepatocellular carcinoma, which would further worsen the haemostatic profile of haemophilic patients (41).

Just like hepatitis, HIV is also inimical to the haemophilic patient, because in addition to causing AIDS with recurrent opportunistic infections, it can cause HIV-associated immune thrombocytopenia (42). Acquisition of any type of immune thrombocytopenia jeopardizes primary haemostasis and aggravates bleeding in haemophilia (43). Therefore, HIV-associated

thrombocytopenia should be treated adequately with anti-retroviral agents in conjunction with immuno-suppressants and/or immune modulators (42). Hence, the haemostatic defects acquired due to viral CLD and/or HIV infection can aggravate the pre-existing haemophilic bleeding tendency, increase bleeding rates and worsen the prognosis of infected haemophiliacs. TTVIs are particularly undesirable in haemophiliacs in tropical developing countries like Nigeria, as they would invariably worsen the prognosis of an already under-treated inherited bleeding disorder. The risk of acquiring TTVIs among haemophiliacs in Nigeria, and indeed other developing countries, can only be prevented by upgrading the National transfusion service and blood safety protocols. This must include efficient donor screening procedures, effective viral inactivation techniques, and local production or regular importation of recombinant blood products, including FVIII concentrates (33). Moreover, it is important that haemophiliacs are regularly and periodically screened for hepatitis and HIV infections so that anti-viral therapy can be started promptly in order prevent the development of 'acquired-on-inherited' bleeding complications.

While effective vaccines have not yet been developed against HIV and hepatitis C infections, hepatitis B infection is preventable through the use of vaccines, and every haemophiliac should be vaccinated in order to thwart the risk of acquiring the virus. Nonetheless, every precaution must be observed in order to avoid the risk of bleeding (44). Thus, subcutaneous administration of vaccines is preferred over intramuscular injection, regardless of disease severity since vaccines delivered by the subcutaneous route are as effective as those injected intramuscularly among haemophiliacs (45). The routine application of ice to the injection site is recommended before and after vaccine administration (44). Compression of the injection site is recommended after vaccination, but rubbing the site of injection should be avoided (44). Needles with the smallest possible gauge should be used whenever haemophilia patients are vaccinated (44). There is insufficient scientific evidence supporting the association between vaccination in haemophiliacs and development of inhibitors against FVIII. Hence, there is no need to avoid vaccination in association with the administration of FVIII concentrate (on the same day) in patients with haemophilia (44), and pre-vaccination FVIII concentrates can be administered, if necessary, to minimize risk of bleeding (45).

Hepatitis A virus is transmitted mostly by faeco-oral route. Nevertheless, it is advocated that hepatitis B vaccine should be complemented with hepatitis A vaccine in haemophiliacs for two reasons (46). First, there had been reports of several outbreaks of hepatitis A among haemophiliac recipients of contaminated FVIII (47). Second, haemophiliacs with chronic hepatitis C may develop severe hepatic decompensation if they become co-infected with hepatitis A (48). Both hepatitis A and B vaccines are non-live vaccines, and can be safely administered to all haemophiliacs, including those with HIV infections (46). Ultimately, it is the responsibility of every country and haemophilia care centre to establish their own vaccination protocols and safety guidelines in accordance with local standards for best healthcare practices.

Cytomegalovirus infection in haemophilia
Unlike HIV, and hepatitis B & C viruses, CMV is usually not routinely screened in clinical blood transfusion practice in most countries despite the fact that CMV is transmissible in blood (29). In fact, CMV is highly contagious because in addition to blood, it is also spread via various other body fluids such as semen, saliva and excrements (29). The virus is known to infect both immune-competent and immune-compromised persons (49). However, while it often causes clinically severe disease in immuno-compromised persons, it usually causes mild disease in immuno-competent individuals (49). The virus has a worldwide distribution. The global blood donor seroprevalence of both CMV-IgG (indicating past exposure with less risk of transmission) and CMV-IgM (indicating recent or active infection with greater risk of transmission) was 83.16% as revealed in a recent meta-analytical study (50). Nonetheless, CMV donor seroprevalence varies from 40–100% in different parts of the world (50). Developed countries tend to have relatively lower prevalence, while developing countries tend to have higher prevalence because of factors such as poor personal and environmental sanitation (50). For example, the seroprevalence of CMV in Nigerian blood donors was reported to be 100% in a previous study (51).

Hence, transfusion-dependent haemophiliacs in developing countries are at high risk of acquiring CMV infection. In another example, a recent study from Iran revealed that more than 70% of multi-transfused haemophiliacs were sero-positive for CMV antibodies (52). While majority of haemophiliacs with normal

immune response may not develop clinically severe CMV disease, it is predictable that CMV would run severe clinical course in three categories of CMV-vulnerable haemophiliacs; first, haemophiliacs with HIV infection (49); second, haemophiliacs who have developed transfusion-associated immune-suppression in the absence of HIV infection (12); and third, haemophiliacs in the neonatal age, which is associated with immature immune system (53). In any case, severe CMV infection is undesirable in haemophiliacs for three reasons. First, CMV may cause mucosal injury, and severe upper and lower gastrointestinal bleeding have been reported even in non-haemophilic patients (54,55); second, CMV infection may cause severe and refractory immune thrombocytopenia, which would worsen any pre-existing gastrointestinal bleeding and trigger other mucocutaneous bleedings even in non-haemophilic patients (56); and third, CMV infection accelerates the progression of HIV infection to full blown AIDS in HIV-infected haemophiliacs (57). Consequently, CMV infection has been shown to increase the overall haemophilic bleeding risks (58) and aggravate immune status of HIV-infected haemophilia patients (57).

It is therefore imperative to conduct post-transfusion haemovigilance on multiply transfused haemophiliacs; any patient presenting with post-transfusion flu-like symptoms in association with undue bleeding should be investigated for CMV infection, which must be promptly treated with appropriate anti-viral medications (59). The prevention of transfusion transmitted CMV in haemophiliacs is more difficult because the staggering seroprevalence of CMV among blood donors, especially in the developing countries, makes it virtually impossible to offer CMV seronegative blood products to haemophiliacs, even for the 'three categories of CMV-vulnerable haemophiliacs'. Fortunately, CMV is a highly leuco-cytotropic virus and leuco-depleted blood is considered relatively CMV-safe irrespective of donor sero-status (60). Therefore, leuco-depletion should have been the best strategy for producing relatively CMV-free blood in the tropics where sero-positivity for CMV antibodies is virtually 100% among donors (51). Regrettably, tropical transfusion services are under developed and cannot readily undertake leuco-depletion of donor blood. Hence, the only feasible method of producing CMV-safe blood in the tropics is by saline washing of red cells, which will wash-off most of the infected donor leucocytes and presumably reduce but not abolish the risk of CMV transmission (61). Unfortunately, 'red cell washing' is not a rational procedure for haemophiliacs scheduled to receive transfusion of 'fresh whole blood' (an easy-to-make blood product that is commonly used to treat haemophilia in low resource tropical countries) because the haemostatically active anti-haemophilic factor (FVIII) is contained within the plasma, which is invariably washed off together with the leucocytes (21).

Haemorrhagic fever viruses

Viral haemorrhagic fevers are caused by seven families of viruses; Ebola, Marburg, Lassa, Dengue, Yellow fever, Crimean-Congo, and Rift Valley fever virus (62). However, to the best of our knowledge based on literature search, only Dengue haemorrhagic fever has been documented in patients with haemophilia.

Dengue haemorrhagic fever in haemophilia

Dengue haemorrhagic fever (DHF) is a mosquito vector-borne (*Aedes aegypti* and *Aedes albopictus*) viral disease caused by the Dengue virus, which belongs to the family filoviridae and genus flavivirus (63). Thrombocytopaenia and hypofibrinogenemia are consistent findings in DHF (64). Hypofibrinogenemia is due to plasma leakage into pleural and peritoneal cavities (i. e., effusions) (64). However, the dominant haemostatic abnormality in DHF is thrombocytopaenia, which is due to the dual effects of myelosuppression and immune mediated platelet destruction (64). Consequently, the haemorrhagic manifestation of DHF range from positive tourniquet test, to ecchymoses, epistaxis, gum bleeding and/or severe gastrointestinal haemorrhages even in non-haemophilic patients (64).

In view of DHF-associated haemostatic derangement, DHF has been reported to aggravate bleeding diathesis among haemophiliacs who are already battling with a pre-existing inherited FVIII deficiency (65). Thus, in addition to FVIII replacement, the management of co-morbid DHF in haemophilia requires the administration of platelet concentrates in cases compounded by severe thrombocytopenia (65, 66). Of special concern is when haemophilic patients with comorbid DHF develop respiratory distress due to pleural plasma effusion (67). In such cases, the effusion should be drained only after good primary and secondary haemostasis are achieved by optimal infusion of platelet and FVIII concentrates (65,66,67), otherwise the pleural effusion may be compounded by haemothorax. Suffices to say that significant pleural effusion, ascites, shock and haemoconcentration signify active plasma leakage

(64,67), which may require supplementation with fresh plasma. In this setting, fresh plasma is of triple clinical value as it would mitigate coagulopathy by replacing any fibrinogen that is lost in leaked plasma, alleviate haemo-concentration by acting as a haemo-dilutor and ameliorate shock by acting as a blood-volume expander (64,67).

Because of the complexities of mana-ging DHF in haemophilia, it is important that haemophiliacs presenting with fever, thrombo-cytopenia and undue bleeding in DHF endemic area should be promptly screened for the infection by both serological and antigen detec-tion methods in order to start early blood products transfusion and other relevant supp-ortive therapies as there is no specific anti-viral therapy at the moment (63). It is paramount for DHF endemic countries to control the spread of the disease through environmental hygiene and vector control programs (63). Moreover, haemophilia care givers should also counsel and encourage haemophiliacs to use insecticides, bed nets and other barrier pro-tection methods, and be vaccinated with the dengue fever vaccine (Dengvaxia) (63). Deng-vaxia is a live-attenuated dengue vaccine (63), hence it cannot be given to HIV-infected persons, including haemophiliacs (46). The vaccine has been shown in clinical trials to be efficacious and safe in persons who have had a previous dengue virus infection (63). However, it carries an increased risk of severe dengue in those who experience their first natural dengue infection after vaccination. Hence, the W.H.O. recommends that only persons aged 9-45 years with evidence of a past dengue infection should receive the vaccine (63). Therefore, only non-HIV-infected haemophiliacs aged 9-45 years, and are living in Dengue endemic areas with a past history of dengue infection are eligible to receive Dengvaxia (63).

Bacterial infections:

Haemophiliacs, especially if immuno-suppressed, may be vulnerable to a myriad of bacterial infections. However, based on litera-ture search, *Helicobacter pylori* and *Myco-bacterium tuberculosis* are the only bacteria that have been specifically associated with IAB in patients with haemophilia.

Helicobacter pylori infection in haemophilia
Helicobacter pylori is a highly ubiqui-tous Gram-negative bacterial pathogen with global distribution and at least half of the world population has been estimated to be infected (68). The bacterium is most likely spread by contaminated food and water via faeco-oral route (69) hence the risk of infection is higher in tropical and developing countries [68]. When *H. pylori* is ingested, it survives the acidity of gastric fluid via urease-mediated production of ammonia, and it subsequently attaches to the gastric epithelium upon which it releases bacterial toxins (70). These toxins cause epith-elial damage and peptic ulceration, which increases the risk of gastrointestinal bleeding even in haemostatically normal persons (70). *H. pylori* infection is therefore undesirable in haemophiliacs for three important reasons. First, *H. pylori* infection significantly raises the risk of upper gastrointestinal bleeding due to peptic ulceration (71). Second, *H. pylori* infec-tion may induce immune thrombocytopenic purpura (ITP), which will aggravate any pre-existing bleeding from peptic ulcers and trigger other mucocutaneous bleeding; hence, *H. pylori*-associated ITP must be treated with immuno-suppressants, but bacterial eradication is the ultimate therapy (72). Third, *H. pylori* infection increases the risk of iron deficiency due to chronic blood loss, especially among haemophiliacs (73).

It is therefore pertinent that haemo-philiacs who present with recurrent symptoms of gastritis and upper gastrointestinal bleeding should be screened for *H. pylori* infection by non-invasive but accurate technique such as the urea breath test (74); and positive cases should be promptly treated with appropriate antibiotics (in combination with anti-ulcer drugs), which is usually in the form of triple or sequential therapy using a proton pump inhibitor in combination with antibiotics (metro-nidazole, amoxicillin, clarithromycin) (75). It must be appreciated that infusion of FVIII-containing blood products alone, without app-ropriate antibiotics, would not be therapeu-tically sufficient in achieving sustainable stop-page of gastrointestinal bleeding in patients with haemophilia, unless the causative micro-organism is eradicated and the ulcer healed with the use of appropriate chemotherapy (75). Moreover, haemophiliacs should be counseled on how to improve environmental, food, water, and personal hygiene in order to prevent faeco-oral acquisition of *H. pylori* in the future. Although there are no vaccines for *H. pylori* infection at the moment, a number of candi-date vaccines are currently in the pipeline of investigations (76).

Mycobacterium tuberculosis infection in haemo-philia
In similarity with the brain, the lungs have high concentration of tissue factor (5),

thus pulmonary haemorrhage and spontaneous haemoptysis are relatively rare manifestations of haemophilia (7,77). Previous studies had shown that whenever haemoptysis is seen in haemophilia, it is usually associated with pulmonary comorbidities such malignancies and/or infections, including TB (7,78). Tuberculosis is a febrile chronic granulomatous inflammatory disease that mainly affects the respiratory tract wherein it causes epithelial damage and tissue cavitations resulting in haemoptysis (79), which can be severe and sometimes life threatening even among non-haemophilic patients (80). As expected, the background inherited FVIII deficiency makes even mild haemophiliacs to be unduly vulnerable to recurrent haemoptysis if and when they acquire pulmonary TB (78).

The risk of acquiring tuberculosis (TB) among haemophiliacs is three-fold. First, the risk of acquiring infectious diseases in general, and TB in particular, is high in Africa and other tropical regions, which carry the heaviest global burden of infectious diseases (15), thus it is not surprising that haemophiliacs in low resource countries are at higher risk of getting TB (81). Second, chronically transfused haemo philiacs often suffer from transfusion-associated immune-suppression (TAIS) (12). TAIS had been shown to increase the susceptibility of haemophiliacs to TB with a significant correlation between the amount of transfused blood products and the development of TB among multi-transfused haemophiliacs (82). Third, chronic transfusion predisposes haemophiliacs to the acquisition of HIV (13), which may progress to AIDS (14), and subsequently predispose to TB (83). It is therefore recommended that HIV infected haemophiliacs should be promptly screened for TB (by using sensitive techniques such as PCR) at the appearance of the earliest indices of suspicion such as fever and cough (84). This will allow prompt case-detection and the commencement of anti-TB chemotherapy in order to avert disease advancement, significant lung damage and life-threatening haemoptysis (84).

Apart from lung injury, another pro-haemorrhagic complication of TB is ITP (85). Tuberculosis-associated ITP can cause severe thrombocytopenia, which would worsen haemoptysis and increase the risk of other muco-cutaneous bleeding episodes. It is therefore important that any haemophiliac with a pre-existing TB who presents with aggravated haemoptysis and/or onset of other muco-cutaneous bleedings in association with thrombocytopenia should be promptly screened for

ITP in order to commence appropriate immuno-suppressive therapy (85).

Acquisition of TB can be effectively prevented by the use of BCG vaccine, which has stood the test of time in terms of efficacy and safety (86). Accordingly, haemophiliacs living in TB endemic countries should be counseled to receive BCG vaccine. However, BCG is a live-attenuated vaccine (87), hence it can only be given to non-HIV-infected haemophiliacs and not to HIV-infected haemophiliacs (46). Nonetheless, unvaccinated HIV-infected haemophiliacs can decrease their risks of contracting TB by maintaining good personal hygiene and optimal nutritional status (88).

Parasitic infections:

Most of the literature on haemophilia originated from non-tropical developed countries, hence only little is known about parasite-induced bleeding and its effect in haemophiliacs. Nevertheless, our literature search revealed that intestinal helminthiasis, urinary schistosomiasis and malaria, and their haemorrhagic effects have been 'scantly' reported among haemophiliacs.

Intestinal helminthiasis in haemophiliacs

Intestinal helminths cause iron deficiency by inducing malabsorption and gastrointestinal haemorrhage (GIH) even in haemostatically normal persons (89). Intestinal helminths, especially the soil-transmitted helminths, cause GIH by inducing mucosal injuries leading to a wide spectrum of chronic or intermittent blood losses that range from occult bleeding to melena, haematemesis, and haematochezia (90-94). In addition to mucosal injuries, some intestinal helminths such hook worms have the capacity to manipulate the host haemostatic system by actively secreting anti-coagulants (anti-FXa and anti-FIXa), which can aggravate bleeding from mucosal ulcers (95). Because intestinal helminthiasis is an important risk factor for GIH even in haemostatically normal individuals, it is easy to infer that haemophiliac would be particularly vulnerable to the haemorrhagic effects of intestinal helminths.

The haemorrhagic impact of intestinal helminths on haemophilia has not been adequately studied because the vast majority of publications on haemophilia arose from developed nations where parasitic diseases are not prevalent. Nevertheless, our literature search revealed only two pertinent studies regarding helminthiasis in haemophilia. The first study was conducted in Nigeria and revealed that up

to 35.7% of haemophiliacs were infected by single or multiple intestinal helminths (*Ancylostoma duodenale, Ascaris lumbricoides* and/or *Trichuris trichiura*) (96), and as expected, infected haemophiliacs had significantly higher frequency of GIH and iron deficiency than their counterparts without helminthiasis (96). The second study was conducted in India and found out that up to 20.4% of haemophiliacs were infected by *Strongloides stercoralis* (97), which in addition to GIH (98), can potentially cause life-threatening disseminated hyper-infection among HIV-infected haemophiliacs when they develop AIDS (99).

These two studies (96,97) have shown that haemophiliacs living in the tropics are at risk of helminthiasis, and because of the pre-existing FVIII deficiency, haemophiliacs would be particularly susceptible parasite-induced haemorrhage and iron deficiency. The findings of these studies have triple clinical implications on haemophilia care in parasite-endemic tropical countries. First, all haemophiliacs presenting with GIH, especially those with past history of passage of worms in stool, should have their stool screened for intestinal helminths, and positive cases should receive specific anti-helminthic chemotherapy (if only one parasite found) or broad spectrum anti-helminthic chemotherapy (if multiple parasites are found) (100). Second, all haemophiliacs should undergo periodic screening and de-worming program in consonance with WHO recommendations for endemic countries (100), and third, haemophiliacs should be counseled to constantly observe personal and environment sanitary measures to prevent re-infections (100).

Urinary schistosomiasis in haemophiliacs

Urinary schistosomiasis, caused by *Schistosoma haematobium*, is a tropical haemorrhagic parasitic disease (15). The adult parasites preferentially settle in the vesical plexus where they reproduce and deposit eggs leading to extensive inflammation, epithelial damage, haematuria and anaemia even in haemostatically normal patients (101). In addition to epithelial injuries, *S. haematobium* parasite is known to secrete serine protease inhibitors with anti-thrombin properties that manipulate the host haemostatic system and escalate the severity of the haematuria (102). It can therefore be easily deduced that haemophiliacs would be particularly vulnerable to the haemorrhagic effects of urinary schistosomiasis.

In similarity with intestinal helminthiasis, there is extreme paucity of studies on urinary schistosomiasis in haemophiliacs even within the literature from tropical countries.

However, a solitary study from Nigeria reported that urinary schistosomiasis was responsible for about 20% of cases of haematuria seen among haemophiliacs (103). Moreover, the study revealed that schistosomal haematuria was severe and associated with significant anaemia in contradistinction to spontaneous haematuria, which was mild and not associated with significant anaemia (103). Therefore, healthcare givers in the tropics should ensure that all haemophiliacs presenting with haematuria in schistosomiasis endemic countries should be properly investigated by urinalysis and blood cell count for early detection and treatment (103). In particular, haemophiliacs who present with apparently spontaneous haematuria in association with dysuria and eosinophilia should evoke the strongest clinical suspicion for urinary schistosomiasis (103). While infected haemophiliacs with active haematuria should be treated with anti-schistosomal chemotherapy, all haemophiliacs in endemic countries should be periodically screening by urinalysis and counseled on personal and environmental sanitary measures to prevent infections (100).

Malaria in haemophiliacs

Malaria is endemic in many tropical countries (15). Five *Plasmodium* species; *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, have been documented to infect humans, with the first two being the most important (15). Haemophiliacs in tropical countries are doubly exposed to the risk of acquiring malaria via mosquito bites and via blood transfusions, because a significant proportion of tropical blood donors have asymptomatic malaria (104). Isolated thrombocytopenia (malarial thrombocytopenia) is a well-known complication of acute malaria (105). The pathophysiologic mechanisms of malarial thrombocytopenia include reduced bone marrow production and/or accelerated peripheral destruction of platelets (106,107,108). Malarial thrombocytopenia, especially in severe cases, can compromise primary haemostasis, and has been reported to cause significant mucocutaneous bleeding (malarial thrombocytopenic bleeding) even in non-haemophilic children and adults (109,110). Hence, malarial thrombocytopenia (MT) and malarial thrombocytopenic bleeding (MTB) are highly undesirable in the haemophiliacs who are already battling with a lifelong congenital bleeding diathesis due to FVIII deficiency.

In similarity with intestinal helminthiasis and schistosomiasis, there is paucity of studies on MTB in haemophiliacs in the lite-

rature, nonetheless, our literature search revealed two pertinent studies. The first study is a case report that was published more than five decades ago (in 1967) by Vartan (111) who described the adverse thrombocytopenic effect of transfusion transmitted malaria due to *P. malariae* in a British patient with haemophilia-B, which is pathophysiologically and clinically very similar to haemophilia-A. The patient was inadvertently transfused with malaria infected red cells from a donor who returned from a malaria endemic country. The second study is a recent retrospective cohort analysis of *P. falciparum* infected haemophiliacs in Nigeria (112). The reported incidence of MTB among haemophiliacs in the study with MT was up to 16.8%. The study further revealed that the risk of MTB was not affected by severity of haemophilia, but the risk was increased in young age (<5 years), and by the severity of parasitemia and thrombocytopenia as well as inheritance of non-O blood groups and HbAA phenotypes (112). It is thus recommended that haemophiliacs presenting with a triad of fever, thrombocytopenia and mucocutaneous bleeding in the tropics should be investigated for MTB, and positive cases should be promptly treated with parenteral anti-malarial chemotherapy and platelet concentrates in addition to FVIII containing blood components (112).

Vaccine-based malaria-prevention strategy remains a high priority for sustained, substantial, and cost-effective malaria control in tropical countries. However, the RTS,S/AS01 vaccine has shown only low to moderate efficacy in preventing clinical *P. falciparum* malaria (113*)*. Although RTS,S/AS01 vaccination alone might not be sufficient for global malaria eradication, it should be considered as another addition to the malaria-control program and not as an eradication tool because of its relatively low to modest efficacy (113). Eventually, in October 2021, the RTS,S/AS01 vaccine was endorsed by the WHO for use in children in conjunction with other malaria-control programs such as insecticide treated nets for barrier protection, and environmental vector control (114). Therefore, haemophiliacs living in *P. falciparum* endemic countries should be counseled to receive RTS,S/AS01 vaccine. The vaccine is a non-live recombinant protein-based vaccine (114) hence it can be given to all haemophiliacs including those with HIV infection (46).

Conclusion and Recommendations:

Haemophiliacs are often transfusion-dependent, and are therefore at risk of HIV and non-HIV immunosuppression, which makes them vulnerable to both transfusion-transmissible infections (TTIs) and non-TTIs, many of which can cause infection-associated bleeding (IAB) even in non-haemophilic individuals. Haemophiliacs are particularly susceptible to IAB due to interplay between pre-existing 'inherited' FVIII deficiency and infection-induced 'acquired' pro-haemorrhagic abnormalities such as mucosal ulcerations, perturbation of synthesis and/or functions of clotting factors, portal hypertension, and /or thrombocytopenia. Hence, IAB in haemophiliacs manifests as undue persistent or recurrent musculoskeletal and/or mucocutaneous haemorrhages. It is thus important for haemophilia caregivers in general (and in the tropics in particular) to have high clinical index of suspicion for IAB in patients whose bleeding is unabated by blood products transfusion alone and/or is accompanied by constitutional or systemic symptoms suggestive of active infection.

The management of IAB requires early identification of the infective agent, followed by dual combination of 'transfusion therapy' with 'anti-infection chemotherapy' in order to achieve prompt and sustained stoppage of IAB. It should be realized that transfusions of FVIII containing blood products without concurrent anti-infection chemotherapy may even promote the development of FVIII inhibitors since active infections and inflammations are important risk factors for inhibitor development in haemophilia. It is also recommended that hemophilia caregivers should offer appropriate counseling on personal and environmental hygiene, barrier protection against disease-spreading vectors, and the role of vaccines in preventing certain types of infections.

Conflict of interest:

Authors declare no conflict of interest

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Review Article   **Open Access**

A review of the roles of Major Histocompatibility Complex (MHC) molecules in infections

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Abstract:

The major histocompatibility complex (MHC) locus is a group of genes located on the short arm of chromosome 6 in human that code for proteins on the cell surface. They have important roles in immune response by the cells of immune system. Using a comprehensive search method on Google Scholar and PubMed databases, literatures on MHC published in English until 2021 were searched with the terms; "MHC", "HLA", "MHC antigen presentation" and "MHC roles in infections". Relevant publications were identified, screened for duplicates and selected per eligibility. The review highlights the different haplotypes of the MHC that either enhance or depress the body immune system to some important viral, bacterial and parasitic infections. The possibility of utilizing this knowledge in genetic engineering and immunomodulation, to prevent infectious diseases and cancers, are discussed.

Keywords: Major histocompatibility complex; human leukocyte antigen; haplotypes; genetic engineering; immunomodulation; review.

Un examen des rôles des molécules du complexe majeur d'histocompatibilité (CMH) dans les infections

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Abstrait:

Le locus du complexe majeur d'histocompatibilité (MHC) est un groupe de gènes situés sur le bras court du chromosome 6 chez l'homme qui codent pour des protéines à la surface des cellules. Ils jouent un rôle important dans la réponse immunitaire des cellules du système immunitaire. À l'aide d'une méthode de recherche complète sur les bases de données Google Scholar et PubMed, les publications sur le CMH publiées en anglais jusqu'en 2021 ont été recherchées avec les termes; "MHC", "HLA", "présentation de l'antigène du CMH" et "rôles du CMH dans les infections". Les publications pertinentes ont été identifiées, examinées pour les doublons et sélectionnées par éligibilité. L'examen met en évidence les différents haplotypes du CMH qui renforcent ou dépriment le système immunitaire de l'organisme contre certaines infections virales, bactériennes et parasitaires importantes. La possibilité d'utiliser ces connaissances dans le génie génétique et l'immunomodulation, pour prévenir les maladies infectieuses et les cancers, est discutée.

Mots clés: Complexe majeur d'histocompatibilité; l'antigène leucocytaire humain; haplotypes; ingénierie génétique; immunomodulation; la revue.

Introduction:

The major histocompatibility complex (MHC) locus is a group of genes that encode proteins on the cell surface that have an important role in immune response (1). The level of immune responsiveness is often affected or controlled by gene products of the MHC, also known in humans as human leukocyte antigens (HLAs) (2). Many diseases, as well as host immune reactivity, are associated with the HLAs (3,4). The MHC were first identified by their potent effect on the immune response to transplanted tissue, reason to which, the gene complex was termed "major histocompatibility complex". The MHC, called the H-2 complex in mice and located on chromosome 17, and on the short arm of chromosome 6p21.31 in humans, has more than 200 genes, and were first recognized in 1937 as a barrier to transplantation in mice (5). The MHC also functions as tissue allorecognition, an important factor in prevention of successful organ transplantation.

The HLAs were first discovered through antigenic differences between white blood cells in different individuals. Their main role is in antigen presentation where they display peptide fragments for recognition by appropriate T cells which is an important process in the immune system response for destroying invading pathogens. The MHC on the cell surface is necessary for cell self-recognition and the prevention of the immune system targeting its own cells. Certain MHC alleles are associated with an increased risk of autoimmune disease such as Hodgkin's lymphoma and multiple sclerosis (1).

The presence of HLA alleles differs in various human populations. Genomic analysis in families has paved way for the mapping and identification of the HLA loci associated with certain infectious diseases and the role they play in conferring resistance or susceptibility on the host. The MHC has two distinct properties which makes evasion of immune response difficult for pathogens. It is polygenic with several different MHC class I and class II genes, such that a set of MHC molecules with different range of peptide-binding specificities is present in every individual. It is also highly polymorphic, making a population of individuals possess multiple variants of each gene.

There is paucity of data on the roles played by the MHCs in infection and against various pathogens. The extent of actions and involvement of this MHC is sparsely understood, thus limiting application of knowledge and use of these molecules in infectious diseases treatment, prevention and control. Also, information on the impact and functions of the MHC in immune responses are sparse.

Hence, this review was conducted to highlight the functions and impacts of the MHC in human, as well as their roles in modulating infections.

Methodology and Results:

In this review, a combination of free-text terms or phrasal terms of "MHC" and/or "HLA", "MHC types", "MHC antigen presentation", "MHC structure" and "MHC roles in infection" were used in carrying out a comprehensive search of literatures through online database search as shown in Fig 1. Articles published in English language up till October 2021, were screened and duplicates removed after determining the relevant articles. This produced 50 articles but further search led to additional 3 articles, giving a total of 53 articles for the review.
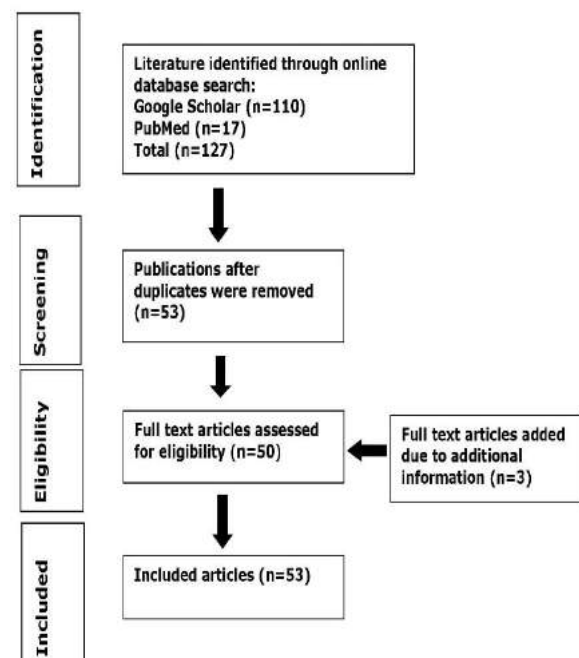
Fig. 1: Process of selection of publications (using PRISMA guide) used for the review

Discussion:

MHC genetic locus

The human major histocompatibility complex (MHC) locus is located on the short arm of chromosome 6 at 6p21.3 (Fig. 2a). Chromosome 6 is estimated to be 150-180 Mb in size and the MHC region on this short arm is a 4-Mb DNA segment that encodes many of the molecules involved in innate and adaptive immune responses. This highly polymorphic DNA region contains nearly 130 genes and approximately 100 pseudogenes but not all of these genes are linked to immunity, and only two sets of genes within the region play central roles in antigen presentation; MHC class I and MHC class II locus

which encode molecules involved in antigen presentation (Fig. 2b). The MHC locus starts at 4954 bp and ends at 3550069 bp.

The MHC locus is made up of three regions; MHC-I, MHC-II, and MHC-III. Each region has specific classical HLA antigens encoded; HLA-A, -B, and -C in the MHC-I region, HLA-DR, -DQ, and -DP in the MHC-II region, while MHC-III region includes several genes involved in the complement cascade (C4a, C4b, C2, and FB), TNF-α and TNF-β (LTa) genes, CYP21 gene that encodes an enzyme in steroid metabolism, HSP70 gene that encodes a chaperone, and many other genes of unknown immunological function. This indicates the important role of MHC in immune-mediated responses, autoimmunity, and infectious diseases (4).

The MHC class I proteins develop a functional receptor on almost all nucleated cells of the body, encoded by 3 major (HLA-A, HLA-B and HLA-C) and 3 minor (HLA-E, HLA-F and HLA-G) MHC class I genes. The genes responsible for encoding α1, α2 and α3-chains of MHC-I and α1, α2 and β1, β2-chains of MHC-II are linked, while the genes for β2-microglobulin in MHC-I and the invariant chain (Ii gene) in MHC-II are located on different chromosomes; chromosomes 15 and 5 in humans, and chromosomes 2 and 18 in mouse respectively.

Fig 2: (**a**) is the major histocompatibility complex locus in a region on the short (p) arm) of human chromosome 6; and (**b**) is simplified genetic map of the MHC regions, showing organizational themes within the MHC locus

Roles of MHC molecules in infections

Afr. J. Clin. Exper. Microbiol. 2022; 23 (2): 120-130
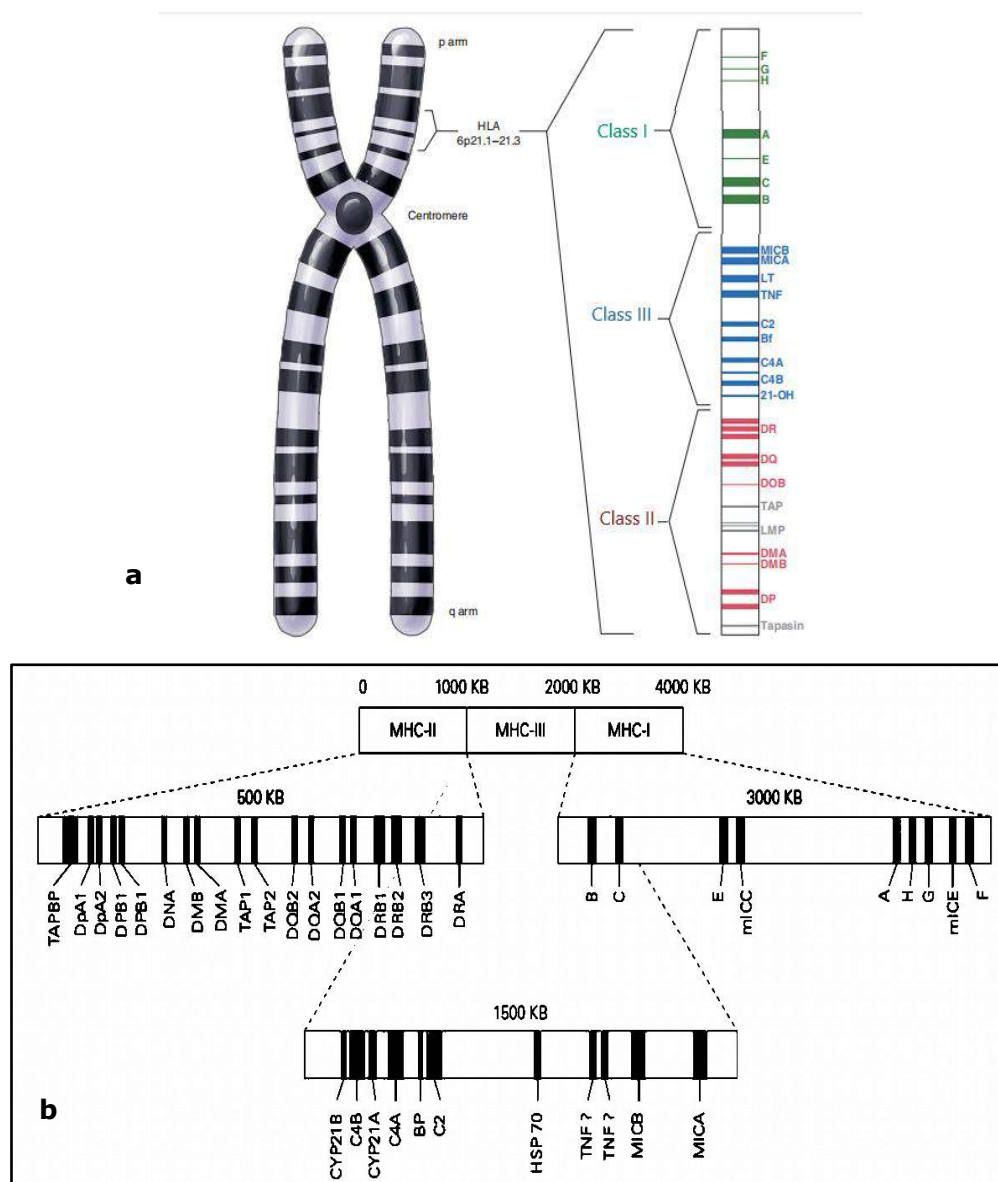
Structure of and factors affecting cellular expression of MHC molecule

There is an interwoven similar relationship in the structures of MHC class I and class II. In the MHC Class I, the binding platform is composed of two domains, originating from a single heavy α-chain (HC), while it originates from two chains (α-chain and β-chain) in the case of MHC class II (Fig. 3). The two domains evolved to form a slightly curved β-sheet, which creates a base and two α-helices at the top, which are wide apart and able to accommodate a peptide chain in-between. The MHC class I molecules are expressed on all nucleated cells, and their classical function is to display peptide fragments of endogenous antigens and present them to cytotoxic CD8^{+} T cells (6).

All MHC-I and MHC-II molecules can present peptides to T cells, but each protein binds a different range of peptides. Although MHC I molecules always function as ligands, reverse signaling was demonstrated two decades ago and plays important roles in cell apoptosis, activation or function. Cross-linking MHC I on T cells triggers Lck, Zap70, and PLCγ1 activation, which leads to T cell activation (7) or apoptosis. In contrast to MHC class II molecules, MHC I molecules have a longer intracellular tail with approximately 40 amino acids, including a tyrosine site (8)

The peptide-binding unit is supported by two membrane-proximal immunoglobulins (Ig), with one Ig domain found in each chain of the class II MHC while in class I MHC, the second Ig-type domain is provided as a result of the non-covalent association of the invariant light chain beta-2 microglobulin (β-2m) with the heavy α-chain. These domains in both MHC I (heavy α-chain) and MHC II (both chains) are anchored by the transmembrane helices (9).

To be stably expressed at the cell surface, the MHC class I molecule consists of trimer of a heavy chain, a light chain (β2 microglobulin), and an antigen peptide bound within the peptide-binding groove (6,9). The MHC class I molecule heavy chain consists of three subunits (α1, α2 and α3 subunits). The α1 subunit contains the heavy chain amino terminus and, combined with the α2 domain, creates the highly polymorphic MHC class I peptide binding groove. These α1 and α2 domains, along with majority of α3 domain, lie in the extracellular space. The α3 domain also contains the transmembrane segment and carboxy terminal portion of the class I heavy chain. The carboxy terminal tail lies within the cytoplasm of the cell. Unlike most other MHC class-I molecules, the carboxy tail of HLA-G is truncated and contains only six amino acids.

The MHC class II molecule is a dimer of two heavy chains, α and β. The α1 and β1 domains of the heavy chains combine to create the class II peptide-binding groove, while the α2 and β2 domains contain the transmembrane segments and the intracellular carboxy-terminal tails (9).
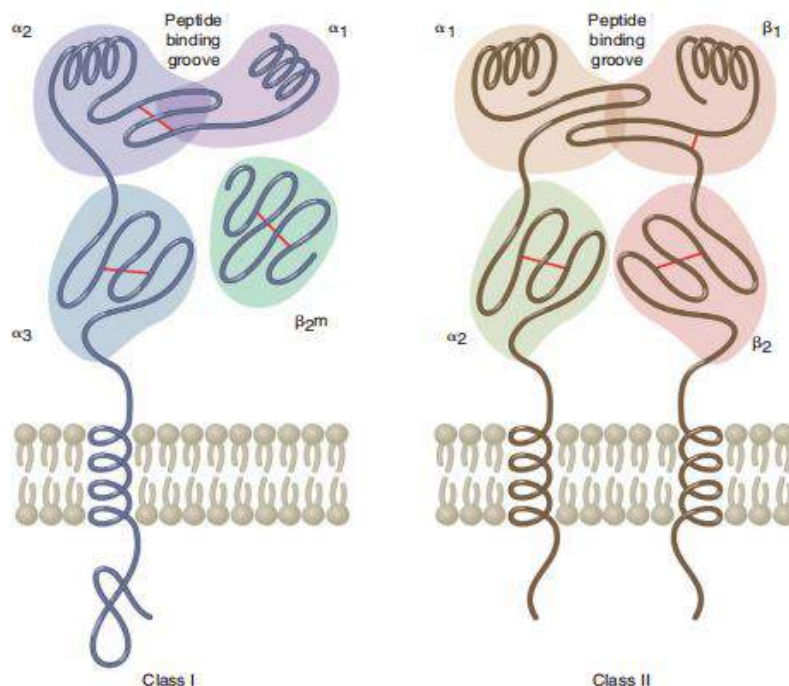
Fig 3: The structures of MHC Class I and Class II, showing the peptide bonding grooves and various helixes

Roles of MHC molecules in infections

Afr. J. Clin. Exper. Microbiol. 2022; 23 (2): 120-130

Antigen presentation by MHC class I and class II molecules

For the purpose of antigen-presentation, MHC Class I and II function specifically for distinct purposes; MHC Class I proteins present foreign peptides to cytotoxic (CD8$^+$) T cells, and MHC Class II proteins present foreign peptides to helper (CD4$^+$) T cells. In general terms, it could be said that the MHC class I and class II molecules are similar in function during antigen presentation in that, they both deliver short peptides to the cell surface allowing these peptides to be recognized by CD8$^+$ (cytotoxic) and CD4$^+$ (helper) T cells respectively. The difference is that the peptides originate from different sources-endogenous or intracellular for MHC class I and exogenous or extracellular for the MHC class II (10).

Cytotoxic T cells recognize foreign peptides in association with class I MHC proteins, whereas helper T cells recognize foreign peptides in association with class II MHC proteins. In both cases, the peptide-MHC complexes are recognized on the surface of an antigen-presenting cell (usually a dendritic cell) or a target cell (Fig 4).
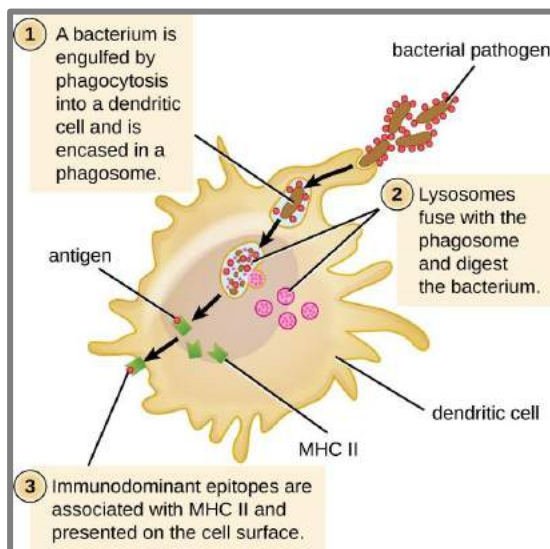
Fig. 4: A dendritic cell and MHC II identifies the most antigenic epitopes from a foreign antigen, select and present on the cell surface for T cells to be recognized and activated

A typical peptide binds in the groove of a class I MHC protein in an extended conformation with its terminal amino group bound to an invariant pocket at one end of the groove and its terminal carboxyl group bound to an invariant pocket at the other end of the groove. Other amino acids (called "anchor amino acids") in the peptide bind to "specificity pockets" in the groove formed by polymorphic portions of the MHC protein (11). The side chains of other amino acids of the peptide point outward, in a position to be recognized by receptors on cytotoxic T cells. Because the conserved pockets at the ends of the binding groove recognize features of the peptide backbone that are common to all peptides, each allelic form of a class I MHC protein can bind a large variety of peptides of diverse sequence. At the same time, the differing specificity pockets along the groove, which bind particular amino acid side chains of the peptide, ensure that each allelic form binds and presents a distinct characteristic set of peptides. Thus, the six types of class I MHC proteins in an individual can present a broad range of foreign peptides to the cytotoxic T cells, but in each individual they do so in slightly different ways.

Class II MHC proteins have a three-dimensional structure that is very similar to that of class I proteins, but their antigen-binding groove does not narrow at the ends, therefore, it can accommodate longer peptides, which are usually 13–17 amino acids long (Fig 5). Moreover, the peptide is not bound at its ends. It is held in the groove by parts of its peptide backbone that bind to invariant pockets formed by conserved amino acids that line all class II MHC peptide-binding grooves, as well as by the side chains of anchor amino acids that bind to variable specificity pockets in the groove (12). A class II MHC binding groove can accommodate more heterogeneous set of peptides than can class I MHC groove. Thus, although an individual makes only a small number of class II proteins, each with its own unique peptide-binding groove, together these proteins can bind and present an enormous variety of foreign peptides to helper T cells, which have a crucial role in almost all adaptive immune responses.

Roles of MHC molecules in infections

Afr. J. Clin. Exper. Microbiol. 2022; 23 (2): 120-130

CYTOTOXIC T CELLS

HELPER T CELLS
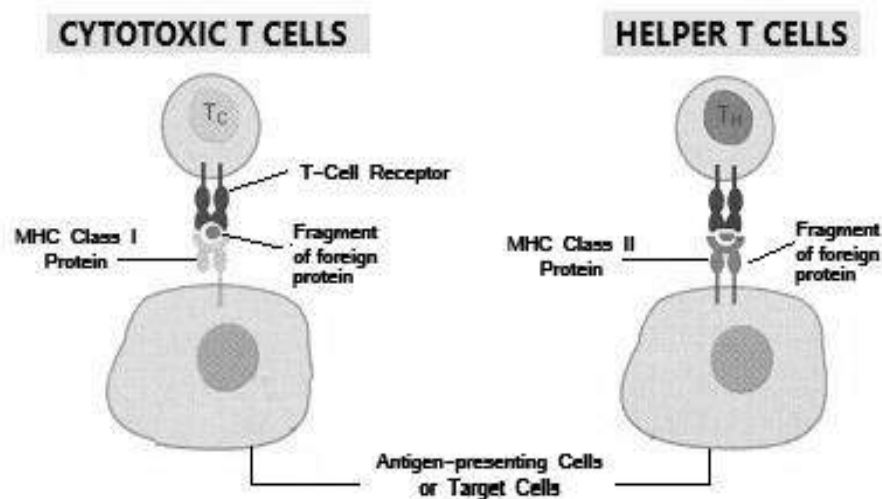
Fig. 5: MHC I and II proteins presenting antigen of foreign peptides to T Cells for recognition

HLA association with infections

Human leucocyte antigen (HLA) alleles and single nucleotide polymorphisms (SNPs) are known to be associated with several infectious diseases and the type of response produced or initiated varies from individuals to individuals, those responses are determined by a lot of factors (13). SNPs in the HLA-B region are likely playing a role in viral suppression during herpes zoster infection, and three SNPs have been identified with infection in HLA-DRA.

MHC and viral infections

The immune system continuously protects its host against pathogens (14). Antigens are often categorized according to whether they are derived from endogenous pathogens (viruses, intracellular bacteria or protozoans) or from exogenous pathogens that replicate outside of the cell. The MHC molecules play a major role in age-related response to infections, as most of the diseases during aging have an immunological pathogenesis associated with the decline of T cell responses and increased propensity to autoimmune reactivity. Intracellular antigens are presented to T cells by any nucleated cell because MHC-I expression is ubiquitous. During viral infections, both innate and adaptive immune cells contribute to effective immune response. T-cells recognize foreign antigens in the form of short peptides that have been processed and displayed on the cell surface bound to MHC-I or MHC-II molecules. In contrast, exogenous antigens are taken up by professional APCs, which process the antigens and present them in the context of MHC-II molecules. An important function of a professional APC, e. g. dendritic cell (DC), is to deliver a second signal (co-stimulation) to

the T-cell to alert it to the presence of an infectious agents

The recognition of peptide-MHC class I (pMHC-I) complexes by CD8$^+$ T cells plays an important role in mediating antiviral immunity (15). CD8$^+$ T cells are essential effectors in antiviral immunity, recognizing short virus-derived peptides presented by MHC class I (pMHC-I) on the surface of infected cells. However, the fraction of viral pMHC-I on infected cells that are immunogenic has not been shown for any virus. There is paucity of studies on the associations of viral diseases with HLA alleles. Nevertheless, in the bid to develop safe and efficient virus vaccines, much work has been done on the mechanisms with which HLA molecules determine the immune response to viral peptides. Since antiviral cytotoxic T-lymphocytes (CTLs) are induced by viral peptides presented within the peptide binding grooves of HLA class I molecules on the surface of infected cells, the aim is to produce virus vaccines that would induce a cellular immune response that leads to the destruction of virus-infected cells by CD8$^+$ CTLs.

The HLA also play an essential role in activation of both natural killer (NK) cells and T cells (13). Natural Killer cell activation is regulated by a variety of activating and inhibitory receptors, including killer-cell immunoglobulin-like receptors (KIRs). KIRs bind to HLA class I molecules, which are expressed on all nucleated cells. As HLA and KIR molecules are highly polymorphic, each individual expresses a unique set of these molecules. The wide range of combinations of HLA and KIR expression results in differences in binding strengths and variations in NK cell activation (16).

The association of HLA and KIR polymorphisms was demonstrated in the experimental simian immunodeficiency virus infec-

tion in rhesus macaques, a model used to study human immunodeficiency virus (HIV) infection (17). Not only do these associations show which interactions contribute to disease resistance, they also pinpoint combinations that increase susceptibility to disease. Such associations of HLA and KIR with disease progression have also been found in hepatitis C virus (HCV) infection (18). In HCV infections, some HLA molecules (e. g. HLA-B27 and HLA-B57) are significantly associated with viral clearance. The force driving these NK cell expansions is largely unclear.

The importance of HLA class I molecule expression levels in host defense using data derived from transporter associated with antigen processing (TAP)-deficient individuals who express less than 10% of normal HLA class I molecules, demonstrate that self-HLA class I molecules shape the KIR repertoire of NKG2C+ NK cells, but are not a requirement for expansion (19). MHC I molecule promotes viral replication independent of suppressing type I IFN production. MHC I molecules are not only key to adaptive CD8+ T-cell responses, but are also involved in the fine-tuning of innate inflammatory cytokine production and antibacterial immunity (15,17,20).

i. HLA association with measles
Infection with measles virus is known to induce a strong T cell response (21), but information regarding the specific measles virus antigens that are responsible for activation of these cells is limited. Schellens et al., (22) investigated which measles peptides are presented by HLA class I molecules by eluting naturally presented peptides from virus-infected cells. They show that a broad spectrum of measles peptidome is presented by different HLA class I molecules.

ii. HLA association with Dengue fever
Heritable factors are likely to be significant in the clinical manifestation of DF such that even in areas of endemicity, only a minute percentage of individuals develop DF or the most severe types of the disease. In an infection caused by DF virus, arrays of genes are associated with an increased synthesis of IFN-γ, IL-8 and IL-10. Investigations on MHC-encoded transporters linked to antigen processing (TAP) genes have also demonstrated interaction with DHF. Furthermore, the study of tumor necrosis factor (TNF) and lymphotoxin alpha (LTA) genes have shown specific combinations of TNF, LTA, and HLA class I alleles which associate with DHF and synthesis of LTA and TNF (23).

iii. HLA association with HCV infection
Many studies have conducted analysis of HLA class I and class II in individuals with hepatitis C virus infection among diverse populations and there is a strong link that some alleles, majorly HLA class II, play a role in the control of HCV infection (24). The most reliable evidence-based data is likely to be related to *HLA-DRB1*11* associated with asymptomatic disease in people with HCV in Italy (*DRB1*11:04* allele) and has been associated with normal serum levels of alanine transaminase (ALT) in infected individuals in France (25). In another separate research in France, *HLA-DRB1*11* has been more commonly found in individuals without cirrhosis when juxtaposed with cirrhotic patients (26).

Across Europe, *HLA-DRB1*11* has been reported to be less common in those people who had received liver transplants for HCV-induced end-stage hepatic disease compared to healthy blood donors. Furthermore, *HLA-DRB1*11* is likely to be a good prognostic indicator, not only in helping spontaneous HCV clearance, but to also increase immunity against progression to more advanced and life-threatening stages of chronic HCV infection (27).

Another allele that has been associated with self-limiting HCV infection is *HLA-DQB1*03* which is found in linkage disequilibrium (LD) with *HLA-DRB1*11* and, standing alone or in partnership with *DRB1*11*, has been strongly linked with spontaneous HCV clearance and with prevention of further hepatic injury in chronically infected HCV patients (28). In multiple scientific studies, people with *HLA-DRB1*11:01* and *HLA-DQB1 *03:01* had minimal predisposition to developing chronic HCV infection in 102% and 136%, respectively. *HLA-DQB1*03* is also likely to influence response to treatment as *HLA-DQB1*03:01* has been linked to sustained viral response (SVR) in infected patients treated with pegylated interferon-α and ribavirin (29). Also, in another study carried out among infected patients in Pakistan, a link between *HLA-DQB1*03* and good antiviral immune response in patients who were treated with interferon-α plus ribavirin was detected (30).

iv. HLA association with HBV infection
A meta-analysis showed that *HLA-DR*03* and *HLA-DR*07* were linked to a high risk of persistent HBV infection in 18 individual case-control studies which included 9 Han Chinese cohorts, 3 Korean cohorts, 2 Iranian cohorts, 1 cohort each of Caucasian, Gambian, Taiwanese, Thai, and Turkish subjects (31). Among the population of Han Chinese, *HLA-DR*01* was linked to clearance of HBV infection, however, in other ethnic groups, there was no link between *HLA-DR*01* and HBV infection (32). The haplotypes *HLA-DQA1*01:02 - DQB1*03:03* and *HLA-DQA1*03:01 - DQB1*06:01* were linked to persistent HBV infection, however, *HLA-*

*DQA1*01:02 - DQB1*06:04* and *HLA-DQA1 *01:01 - DQB1*05:01* provided protection against HBV infection (32).

A genome-wide association study (GWAS) revealed a significant correlation of chronic hepatitis B in Asians with 11 SNPs in a region including *HLA-DPA1* and *HLA-DPB1* which subsequent analyses identified risk haplotypes; *HLA-DPA1*02:02 - DPB1*05:01* and *HLA-DPA1*02:02 - DPB1*03:01* and protective haplotypes; *HLA-DPA1*01:03 - DPB1 *04:02* and *HLA-DPA1*01:03 - DPB1*04:01* for HBV infection (33). Further analysis of *HLA-DQA1* and *HLA-DQB1* haplotypes showed that *HLA-DQA1*01:02-DQB1*03:03* and *HLA -DQA1*03:01-DQB1*06:01* were risk types for persistent HBV infection while the *HLA-DQA1 *01:02-DQB1*06:04* and *HLA-DQA1*01:01-DQB1*05:01* were protective haplotypes for HBV infection (33).

v. HLA association with HIV infection

Several of disease-protective and disease-susceptible HLA alleles have been well described in HIV infection and the greatest link is likely to be related to HLA class I alleles (majorly HLA-A and B alleles) with variable rates of HIV disease outcome (34). The virologic and immunologic outcomes in individuals with HIV infection can be highly different, with only a few infected individuals capable of controlling viral replication without institution of treatment (35).

Previous studies showed a relationship between *HLA-B*27* and *HLA-B*57* with slower progression and development to AIDS. Ever since, other studies have explored the importance of HLA class I and class II alleles in both acute and chronic HIV infection and the greatest link was identified to be related to HLA class I alleles (36). Related to the association of HLA class I alleles and protection against HIV infection, *HLA-B*44* and *HLA-B*57* have been identified as favorable factors in both the acute and chronic phases of sub-Saharan Africans who are seroconverts. In the Peoples Republic of China, *HLA-A*03* has been identified as a preventive factor against HIV-1 infection as well as disease progression (37). Also, in a study in the USA, *HLA-A*32, HLA-A*74, HLA-B*14, HLA-B*45, HLA-B*53,* and *HLA-B*57* have been associated with disease control in Americans of African descent who are infected by HIV-1 subtype B (38).

In a cohort study among a multi-ethnic population with HIV-1 controllers and progressors, it was reported that there are various alleles associated with virologic and immunologic control of the infection; *HLA-B*57:01, HLA-B*27:05, HLA-B*14/C*08:02, HLA-B*52,* and *HLA-A*25*. In addition, *HLA-B*13:02* and *HLA-B*58:01* have been identified as good prognostic indicators (39). In as much as all these alleles are likely to play a role in HIV infection, the most reliable information is linked to three HLA-B specificities; *HLA-B*57* (*HLA-B*57:01* in the European population, and *HLA-B*57:02* and *HLA-B*57:03* mainly in the African population), *HLA-B*27* (*HLA-B*27:05*), and *HLA-B*81* (*HLA-B*81:01*) (39). These alleles are closely related with viral load control as well as delayed disease progression among the various populations. More so, the HLA-B molecules have a significant influence on HIV infection as the many of detectable HIV-specific CD8+T-cell responses identified is likely to be restricted by HLA-B alleles (40).

Concerning HIV susceptibility and the rapid disease progression, *HLA-B*35* (*HLA-B* *35:01, HLA-B*35:02* and *HLA-B*35:03*) is likely to have the highest effect on HIV disease. Individuals who have these alleles seem to have poorer control of viral replication and progress towards AIDS faster (41). Other alleles associated with poor prognosis have been identified and include; *HLA-B*18/*18 :01*, *HLA-B*45/*45:01*, *HLA-B*51:01*, *HLA-B*53:01*, *HLA-B*58:02*, *HLA-A*36:01*, and *HLA-B*07:02* in no specific order (41).

vi. HLA association with HPV infection

A few heritable predisposing factors to the development of cancer following HPV infection have been enumerated, and chief among them is the HLA complex, which performs a major role in susceptibility to cervical carcinoma. Since the first report of a correlation of *HLA-DQ3* with cervical carcinoma (42), a lot of studies of HLA correlation with cervical carcinoma have been peer reviewed and published with different results based on the ethnic group. One of such studies showed that *HLA-DRB1*04:07-DQB1 *03:02* as well as *HLA-DRB1*15:01-DQB1 *06:02* were closely linked to susceptibility to HPV-16 positive invasive carcinoma of the cervix, high squamous intraepithelial lesion (HSIL), as well as carcinoma in situ (43). In Honduran women it was determined that *HLA-DQA1*03:01* in linkage disequilibrium with all HLA-DR4 subtypes in Mestizos, has a greater risk of having high squamous intraepithelial lesion as well as cervical carcinoma (44).

A few *HLA-DR-DQ* haplotypes containing *DQB1*03:01* have been directly correlated with susceptibility to cervical carcinoma; *DRB1*11:01-DQB1*03:01* among Senegalese population and American whites. *HLA-DRB1*11:02-DQB1*03:01* was also commoner among Hispanics with carcinoma *in situ* or HSIL (44). Immunity has been closely associated with *HLA-DRB1*13* group; *HLA-DRB1*13:01* in individuals from Costa Rica, and *HLA-DRB1*13:01-DQB1*06:03-DQA1*01:03* among Swedish, French and Dutch adult

females with cervical carcinoma (44). A protective effect against the progression of malignancy of the cervix has also been correlated with *HLA-DQB1*05, HLA-DQA1*01:01 /04, HLA-DRB1*01:01* and *HLA-DRB1*13:02* among Brazilians (44)·

In the Caucasians, *HLA-DRB1*13* and HPV-16/18-negative status, have been independently associated with an increased probability of regression of low squamous intraepithelial lesion (LSIL), also indicating a protective effect against the progression of cervical carcinoma (44)· Also, in individuals who have cervical carcinoma, the predisposing risks differed between HPV positive and negative cases for several alleles; a higher risk of cervical cancer was seen in patients with *HLA-DRB3(52)*02/03* and *HLA-DRB1*3 (17)- DRB1*3(17)* while a reduced risk was observed with *HLA-DRB1*09:012* and *HLA-DRB5 (51)*01/02* (44).

HLA and parasitic diseases

i. HLA association with Chagas disease

There is a common belief that during *Trypanosoma cruzi* infection, the host immune mechanism induces complex processes to enhance the control of parasite growth. The immune response is important for defense against the disease however, immunological disequilibrium can lead to heart and digestive tract lesions in infected individuals. The spectrum of clinical manifestation of Chagas disease highly suggests the influence of the genetic factors on the clinical progression of the disease, and the variations of genes involved in both the innate as well as the specific immune response is being extensively researched, like the molecules and genes in the region of the HLA (45).

Both the polymorphic HLA classes I (A, B and C) and II (DR, DQ and DP) molecules influence the efficiency of presentation of the *T. cruzi* epitopes to CD8$^+$ and CD4$^+$ T-cells respectively. The type of the presentation is likely to affect the clinical course of diseases because infected individuals are likely to respond in different ways to the same antigen, depending on their HLA repertoire. Many HLA alleles and haplotypes are known to be associated with Chagas disease (46).

Concerning the link between HLA and Chagas disease, *HLA-Dw22* was first linked to the risk of developing the disease in infected people in Venezuela. Another study further compared class II allele frequencies between patients and controls and this study found a reduced frequency of *HLA-DRB1*14* and *HLA-DQB1*03:03* in infected individuals, indicating protective effects not associated with persistent infection in this population (47).

In southeastern Brazil, a study demonstrated that *HLA-A*30* confers risk of contracting Chagas disease, while the *HLA-DQB1 *06* confers immunity notwithstanding the clinical stage of the disease. Also, HLA-DR2 antigens were associated with increased risk of contracting persistent Chagas disease in a South Brazilian population (48). HLA-DR4 and HLA-B39 were linked to the disease in Mexico and *HLA-DRB1*04:09* and *HLA-DRB1 *15:03* in a population in Argentina (48). Another study showed that *DRB1*11:03* allele was associated with immunity against Chagas disease. *HLA-DRB1*14-DQB1*03:01* haplotypes were associated with immunity against *T. cruzi* infection in the rural population in southern part of Peru (49). *HLA-DR B1*01-B*14-MICA*011* haplotypes have been linked to immunity against persistent Chagas disease in Bolivia (50).

ii. HLA association with malaria

A strong link between the HLA class I (*HLA-B53*) and immunity against life threatening forms of malaria has been clearly elucidated, and said to be regulated by HLA class I restricted CTLs during the hepatic stage of the parasite's life cycle (50). Certain genes on the HLA complex possibly protect individuals in endemic regions against the serious forms which are caused by both *Plasmodium falciparum* and *Plasmodium vivax* (13)*.

The antibody mediated immune response that occurs in malaria infections is of special interest, because the production of specific IgG antibodies is mandatory for acquired immunity. However, variations in antibody responses could result from multiple genetic types of the HLA class II molecules. With more concentration on the production of subunit vaccines, studies of the role of class II alleles in the immune defense response in ethnically different populations is critical, before the implementation of vaccine trials.

It has been demonstrated that the *HLA-DRB1*04* alleles were linked to an increased frequency of humoral responses to five out of the nine recombinant proteins tested in Brazil, Nigeria and Ghana (51,52). In Mumbai, India, a study showed that *HLA-B49* and *HLA-DRB1*08:09* were directly associated with complicated severe malaria (53). Contrastingly, *HLA-A19*, *HLA-B5* and *HLA-B13* provided protection against malaria in infected individuals with high parasite index (>2%). The results from this study indicate the significance of ethnicity, which should be considered when producing ideal malaria vaccine (53).

Conclusion:

This review shows that there is a cor-

Roles of MHC molecules in infections

Afr. J. Clin. Exper. Microbiol. 2022; 23 (2): 120-130

relation of HLA-DQ haplotypes with cervical carcinoma and primary liver cell cancer. It could therefore be postulated that genetic modification of HLA genes associated with increased susceptibility to infections by HPV and HBV could be useful in preventing the development of cancers resulting from such infections. Such haplotypes include, but not limited to *HLA-DRB1*04:07 - DQB1*03:02, HLA-DRB1*15:01 - DQB1*06:02* and *HLA-DQ B1*03:01,* which have been directly correlated with susceptibility to cervical carcinoma. Haplotypes *HLA-DQA1*01:02 - DQB1* 03:03* and *HLA-DQA1*03:01 - DQB1*06:01* were also linked to persistent HBV infection and primary liver cell carcinoma. In addition, a strong link has been described between HLA class I (*HLA-B53*) and immunity against life threatening forms of malaria. The gene products of *HLA-B53* should be considered for use in immunomodulation to prevent severe forms of malaria such as cerebral malaria and nephrotic syndrome among children in malaria endemic zones.

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Roles of MHC molecules in infections

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Procalcitonin as a biomarker of adult bacterial sepsis

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Original Article   **Open Access**

Evaluation of procalcitonin as a biomarker of bacterial sepsis in adult population in a tertiary healthcare facility in Lagos, Nigeria

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Abstract:

Background: Prompt antibiotic treatment of sepsis improves the outcome, but dependence on clinical diagnosis for empiric therapy leads to overuse of antibiotics which in turn promotes the emergence of antibiotic resistance. Blood culture takes time and molecular diagnosis may not be available or affordable. The use of procalcitonin (PCT) as a biomarker to guide antibiotic therapy in adults is less established compared to children. This study was therefore designed to evaluate the usefulness of PCT as a biomarker to aid early commencement of antibiotics among adult patients with sepsis in a tertiary healthcare facility in Lagos, Nigeria.
Methodology: Three hundred patients with clinical diagnosis of sepsis made by the managing physicians were recruited for the study. Criteria used for clinical diagnosis of sepsis include tachycardia, tachypnea, fever or hypothermia and presence of leukocytosis, bandemia or leucopenia. The patients were selected using systematic consecutive sampling methods. A sepsis work-up including quick sequential organ failure assessment (qSOFA), white blood cell count (WCC), aerobic blood culture and estimation of serum PCT levels were done for all the participants. Data were analysed using the Statistical Package for Social Sciences (SPSS) for windows version 25.0. Sensitivity, specificity, positive, and negative predictive values, accuracy and likelihood ratio of PCT against blood culture, WCC and qSOFA score were determined. Association between variables was measured using Fisher exact test (with Odds ratio and 95% confidence interval). P-value <0.05 was considered statistically significant.
Results: There were 127 (42.3%) males and 173 (53.7%) females with the mean age of 44.9±14.5 years. Majority (96.2%, n=75/78) of the patients who were culture positive for bacterial pathogens had PCT level ≥10ng/ml, which showed statistically significant association of bacteraemia with PCT level (OR=1362.5, 95% CI=297.9-6230.5, *p*<0.0001). At PCT cut-off value of 0.5ng/ml, the negative predictive value of 100% almost confirms absence of systemic bacterial infection. The high sensitivity, specificity, positive predictive value, negative predictive value, accuracy and likelihood ratio of 94.9%, 98.6%, 96.2%, 98.2%, 97.7%, and 69.9 respectively recorded at PCT level of 10ng/ml indicates that this cut-off level is strongly diagnostic of systemic bacterial infection.
Conclusion: In this study, we observed that PCT levels were significantly higher in patients with positive culture (bacteraemia) and PCT was able to differentiate bacterial sepsis from non-bacterial infections. The findings of this study support the usefulness of PCT as a biomarker for early diagnosis of systemic bacterial infections in adult patients.

Keywords: procalcitonin; biomarker; sepsis; sequential organ failure assessment; adults; evaluation

Évaluation de la procalcitonine en tant que biomarqueur de la septicémie bactérienne chez la population adulte dans un établissement de soins de santé tertiaires à Lagos, au Nigeria

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Abstrait:

Contexte: Un traitement antibiotique rapide de la septicémie améliore les résultats, mais la dépendance au diagnostic clinique pour le traitement empirique conduit à une surutilisation des antibiotiques qui à son tour favorise l'émergence de la résistance aux antibiotiques. L'hémoculture prend du temps et le diagnostic moléculaire peut ne pas être disponible ou abordable. L'utilisation de la procalcitonine (PCT) comme biomarqueur pour guider l'antibiothérapie chez l'adulte est moins établie que chez l'enfant. Cette étude a donc été conçue pour évaluer l'utilité de la PCT en tant que biomarqueur pour faciliter le début précoce des antibiotiques chez les patients adultes atteints de septicémie dans un établissement de soins de santé tertiaire à Lagos, au Nigeria.

Méthodologie: Trois cents patients avec un diagnostic clinique de septicémie posé par les médecins traitants ont été recrutés pour l'étude. Les critères utilisés pour le diagnostic clinique du sepsis comprennent la tachycardie, la tachypnée, la fièvre ou l'hypothermie et la présence d'une leucocytose, d'une bandémie ou d'une leucopénie. Les patients ont été sélectionnés à l'aide de méthodes d'échantillonnage consécutifs systématiques. Un bilan de septicémie comprenant une évaluation séquentielle rapide des défaillances d'organes (qSOFA), une numération des globules blancs (WCC), une hémoculture aérobie et une estimation des taux sériques de PCT a été effectué pour tous les participants. Les données ont été analysées à l'aide du package statistique pour les sciences sociales (SPSS) pour Windows version 25.0. La sensibilité, la spécificité, les valeurs prédictives positives et négatives, la précision et le rapport de vraisemblance de la PCT par rapport à l'hémoculture, le WCC et le score qSOFA ont été déterminés. L'association entre les variables a été mesurée à l'aide du test exact de Fisher (avec rapport de cotes et intervalle de confiance à 95 %). La valeur P <0,05 a été considérée comme statistiquement significative.

Résultats: Il y avait 127 (42,3%) hommes et 173 (53,7%) femmes avec un âge moyen de 44,9±14,5 ans. La majorité (96,2%, n=75/78) des patients dont la culture était positive pour les agents pathogènes bactériens avaient un taux de PCT ≥ 10ng/ml, ce qui a montré une association statistiquement significative de la bactériémie avec le taux de PCT (OR=1 362,5, IC à 95%=297,9-6230.5, *p*<0.0001). À la valeur seuil PCT de 0,5ng/ml, la valeur prédictive négative de 100% confirme presque l'absence d'infection bactérienne systémique. La sensibilité élevée, la spécificité, la valeur prédictive positive, la valeur prédictive négative, l'exactitude et le rapport de vraisemblance de 94,9%, 98,6%, 96,2%, 98,2%, 97,7% et 69,9 respectivement enregistrés au niveau PCT de 10 ng/ml indiquent que cette réduction- hors niveau est fortement le diagnostic d'une infection bactérienne systémique.

Conclusion: Dans cette étude, nous avons observé que les taux de PCT étaient significativement plus élevés chez les patients ayant une culture positive (bactériémie) et que la PCT était capable de différencier la septicémie bactérienne des infections non bactériennes. Les résultats de cette étude confirment l'utilité de la PCT en tant que biomarqueur pour le diagnostic précoce des infections bactériennes systémiques chez les patients adultes.

Mots clés: procalcitonine; biomarqueur; état septique; qSOFA; adultes; évaluation

Introduction:

Sepsis is an important cause of mortality worldwide resulting in 210,000 deaths annually in the United States of America (1). An audit revealed a high prevalence of sepsis, with 53% mortality in Nigeria especially in people with co-morbidities such as diabetes mellitus, chronic kidney disease, and acquired immune deficiency syndrome (AIDS) (2). Prompt and appropriate antibiotic administration enhances good clinical outcome. The consensus guidelines recommend antibiotic therapy within one hour of making a diagnosis of "suspected sepsis" and the choice of empiric antibiotic therapy is usually based on the focus of infection, history of recent antibiotic use, rise in inflammatory biomarkers such as C-reactive protein (CRP) or procalcitonin (PCT), and local resistance pattern (3,4).

Isolation of the pathogen from culture is regarded as the "gold standard" for diagnosis of severe bacterial infections and sepsis (5). There are however, limitations to early diagnosis of severe bacterial infection which include diagnostic delays and low sensitivity in blood culture methods. It has also been shown that a delay in antibiotic therapy is associated with increase in morbidity and mortality especially in patients with septic shock (6). Nonetheless, over-prescription and inappropriate use of empiric antibiotics contributes to the emergence of antibiotic resistance (7). In USA, South Africa and Nigeria, reports showed that doctors over-prescribe antibiotics in up to 41%, 54%, and 75.5% of the time, respectively, leading to the

development of resistance (2,8). The study of antibiotic overuse in patients with suspected sepsis revealed that 29% of the patients received antibiotics for minimum of seven days despite the absence of bacterial disease from laboratory investigations (9). In another study (2), it was reported that all the patients with diagnosis of suspected sepsis received empiric antibiotics and further evaluation revealed that 24.5% were inappropriate while only 12.5% had culture results to guide definitive antibiotic therapy.

In this diagnostic dilemma, PCT has given much hope as a more specific biomarker for bacterial infection. Procalcitonin is a pre-hormone of calcitonin secreted by the C-cell of the thyroid in response to hypercalcaemia and negligible serum PCT concentrations are seen under healthy condition (10). There is however a significant rise in the level of procalcitonin in bacterial infections with systemic inflammation. Procalcitonin increases within two to four hours of bacterial infection, peaks at four to twenty hours, and falls when the infection is controlled. In viral infections, PCT concentration is normal whereas CRP takes 24 hours to rise, and is also elevated in viraemia. Procalcitonin level remains high as long as the bacterial infection persists which makes it suitable for diagnosis, monitoring prognosis, and offering guidance for appropriate antibiotic use in the treatment of bacterial infections (11,12).

In Nigeria, PCT has been reported to be useful in diagnosis of neonatal sepsis (13). Other studies have also shown that serum PCT helps clinicians to differentiate between typical bacterial and non-bacterial causes of lower respiratory tract infections (14). Elevated serum PCT concentration is both sensitive and specific in distinguishing bacterial from non-bacterial causes of sepsis, and also useful in monitoring the severity of bacterial infections. The level of PCT can be readily measured in the blood requiring little expertise and can be done in the absence of power supply. Procalcitonin guided management of severe bacterial infections reduces not only total antibiotic use but also emergence of antibiotic resistance (15, 16).

A national survey of antimicrobial prescribing in Nigeria reported very low utilization of biomarkers, with only about 0.5% across the four tertiary hospitals studied (17). This survey subsequently highlighted the need to use PCT as a guide for empiric antimicrobial therapy (17). This study was therefore designed to evaluate diagnostic use of PCT as a tool for differentiating bacterial from non-bacterial causes

of sepsis among adult patients in a tertiary healthcare facility in Lagos, Nigeria.

Materials and method:

Study area and design

This was a cross sectional study of 300 adult patients with clinical sepsis at the medical emergency unit and wards of Lagos University Teaching Hospital (LUTH), Lagos, Nigeria between May 2019 and April 2020.

Ethical approval

Ethical clearance was obtained from the LUTH Health Research Ethics Committee and a written consent was also obtained from all the participants.

Study population and selected participants

Patients with clinical diagnosis of sepsis made by the managing physicians were recruited for the study using systematic consecutive sampling method. A case of sepsis was defined as life threatening organ dysfunction caused by a dysregulated host response to infection (18). The clinical screening of all the patients recruited for the study was based on quick sequential organ failure assessment (qSOFA) score, which include criteria of; alteration in mental status, systolic blood pressure ≤ 100 mmHg and respiratory rate ≥ 22/min in a patient with suspected infection. The score of 2 or more was considered as suspected sepsis with high risk of mortality (18,19).

Patients with other medical conditions that can interfere with the result of PCT were exempted from the study for example patients with ischemic bowel disease, medullary thyroid cancer, burns, post-operative complications and cardiogenic shock.

Diagnostic assessment of selected participants

A diagnostic sepsis workup which comprised white cell count (WCC), blood culture, and measurement of serum procalcitonin level were done for all selected patients. In patients with identifiable focus of infection, appropriate specimens such as sputum, pus and urine were also collected for microscopy, culture and sensitivity testing (result of this has been presented in another manuscript yet to be published).

Sample processing

Blood PCT concentration was determined using immuno-chromatographic test (ICT) kit (Liming Bio) according to the manufacturer's guidelines for the test procedure and interpretations (13,19,20).

Blood samples were cultured in BACTEC 9050 automated blood culture system. The incubation temperature was 35-37℃ and when the culture bottles were flagged for growth or after five days of incubation, the bottles were removed from the system. Samples were taken from positive bottles and sub-cultured on Mac-Conkey and 5% sheep blood agar plates. Blood agar plate was incubated in 5% CO_2 condition and MacConkey agar plate was incubated in ambient air for 16 to 24 hours.

Isolates were identified using standard laboratory procedures including colony morphology, Gram stain reaction, motility, and Micro-bact identification kit. Other specimens were analyzed using conventional standard laboratory protocols and the Clinical and Laboratory Standard Institute (CLSI) guideline (21).

Data analysis

The data collected were entered into Excel sheet and analysed using the International Business Machine Statistical Package for the Social Sciences (IBM SPSS) for windows version 25.0 (IBM Corp., Armonk, New York, USA). The data were presented in frequency tables and summary statistics. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and likelihood ratio of PCT against blood culture, WCC and qSOFA score were determined. Association between variables was measured using Fisher exact test (with Odds ratio and 95% confidence interval) and p-value < 0.05 was considered statistically significant.

Results:

Demographic and baseline characteristics of the study participants

The selected participants comprise 127 males (42.3%) and 173 females (53.7%) with age range from 20 to 82 years and mean age of 44.9±14.5 years. Among the underlying disease conditions identified from the selected participants, diabetes (24.3%) and HIV/AIDS (14.3%) were the most common co-morbidities (Table 1).

Bacterial culture positivity among participants

Of the 300 participants, 79 (26.3%) were culture positive while 221 (73.7%) were culture negative. *Staphylococcus aureus* (29.1 %, n=23) was the most frequent pathogen isolated from culture, followed by *Klebsiella pneumoniae* (19.0%, n=15) and *Escherichia coli* (16.5%, n=13) (Table 2).

Table 1: Demographic and baseline characteristics of the study participants

Characteristics	Frequency (%)
Gender	
Male	127 (42.3)
Female	173 (57.7)
Age group (years)	
20-29	48 (16.0)
30-39	75 (25.0)
40-49	60 (20.0)
50-59	68 (22.7)
60-69	32 (10.7)
≥70	17 (5.7)
Mean±SD	44.93±14.5
Co-morbidities	
Diabetes mellitus	73 (24.3)
HIV/AIDs	43 (14.3)
Heart failure	26(8.7)
Chronic renal failure	19 (6.3)
Malignancy	15 (5.0)
Others	22 (7.3)

qSOFA= quick sequential organ failure assessment; HIV/AIDS =human immunodeficiency virus/ acquired immune deficiency syndrome

Association of serum PCT levels with culture positivity

All the patients with PCT below 0.5 ng/ml (100%, n=121) and those with PCT level ≥0.5 to <2ng/ml (100%, n=47) were culture negative for bacterial pathogens, while only 7.4% (n=4) of the 54 patients with PCT level ≥ 2ng/ml to <10 ng/ml had bacterial growth. Majority (96.2%, n=75) of the patients with serum PCT level ≥10 ng/ml (n=78) were bacterial culture positive.

The different categories of PCT values were significantly associated with blood culture positivity or negativity (p<0.001) (Table 3). However, 9 (3.0%) of the 300 participants had contaminants isolated from their blood cultures and their PCT values were less than 0.5 ng/ml. All the positive culture samples had the same bacteria species isolated from two blood culture bottles.

Table 2: Bacterial pathogens isolated from the clinical specimens of selected patients

Isolate	Blood culture (%)	Urine (%)	Sputum (%)	Wound specimen (%)	Pus aspirate (%)	Ascitic fluid (%)	Total (%)
Gram-positive cocci							
Staphylococcus aureus	23 (29.1)	1 (4.5)	4 (14.8)	11 (32.4)	2 (40.0)	1 (100.0)	42 (25.0)
Coagulate negative staphylococci (CoNS)	7 (8.8)	6 (27.3)	0	3 (8.8)	1 (20.0)	0	17 (10.1)
Enterococcus faecalis	1(1.3)	0	0	0	0	0	1 (0.6)
Streptococcus pneumoniae	0	0	1 (3.7)	0	0	0	1 (0.6)
Enterococcus faecium	0	1 (4.5)	0	0	0	0	1 (0.6)
Sub-total	**31 (39.2)**	**8 (36.4)**	**5 (18.5)**	**14 (41.2)**	**3 (60.0)**	**1 (100.0)**	**62** (36.9)
Gram-negative bacilli							
Escherichia coli	13 (16.5)	7 (31.8)	6 (22.2)	3 (8.8)	1 (20.0)	0	30 (17.9)
Klebsiella pneumoniae	15 (19.0)	1 (4.5)	9 (33.3)	3 (8.8)	0	0	28 (16.7)
Pseudomonas aeruginosa	2 (2.5)	1 (4.5)	0	6 (17.6)	0	0	9 (5.4)
Klebsiella oxytoca	3 (3.8)	0	3 (11.1)	2 (5.9)	0	0	8 (4.8)
Acinetobacter baumannii	3 (3.8)	2 (9.0)	0	2 (5.9)	0	0	7 (4.2)
Enterobacter aerogenes	2 (2.5)	1 (4.5)	2 (7.4)	0	0	0	5 (3.0)
Proteus mirabilis	2 (2.5)	0	0	1 (2.9)	1 (20.0)	0	4 (2.4)
Citrobacter koseri	0	0	2 (7.4)	1 (2.9)	0	0	3 (1.8)
Serratia marcescens	2 (2.5)	1 (4.5)	0	0	0	0	3 (1.8)
Acinetobacter lwoffii	2 (2.5)	1 (4.5)	0	0	0	0	3 (1.8)
Providencia rettgeri	2 (2.5)	0	0	0	0	0	2 (1.2)
Citrobacter freundii	1 (1.3)	0	0	1 (2.9)	0	0	2 (1.2)
Enterobacter agglomerans	1 (1.3)	0	0	0	0	0	1 (0.6)
Pseudomonas fluorescens	0	0	0	1 (2.9)	0	0	1 (0.6)
Sub-total	**48 (60.8)**	**14 (63.6)**	**22 (81.5)**	**20 (58.8)**	**2 (40.0)**	**0**	**106 (63.1)**
Grand total	**79 (47.0)**	**22 (13.1)**	**27 (16.1)**	**34 (20.2)**	**5 (2.9)**	**1 (0.6)**	**168 (100)**

Table 3: Univariate analysis of association of categories of serum PCT levels with blood culture positivity

PCT values (ng/ml)	Culture positive (%) (n=79)	Culture negative (%) (n=221)	Total (%)	OR (95% CI)	p-value
< 0.5ng	0	121 (100.0)	121 (100.0)	0.005 (0.003 - 0.085)	<0.0001*
≥ 0.5 - <2	0	47 (100.0)	47 (100.0)	0.023 (0.001 - 0.379)	<0.0001*
≥ 2.0 - <10	4 (7.4)	50 (92.6)	54 (100.0)	0.182 (0.063 - 0.523)	0.0003*
≥ 10	75 (96.2)	3 (3.8)	78 (100.0)	1362.5 (297.9 - 6230.5)	<0.0001*

PCT= Procalcitonin: OR = Odds Ratio; CI = Confidence Interval; * = statistically significant

Diagnostic parameters of PCT at different cut-off values

The cut-off values of PCT were set at 0.5ng/ml, 2ng/ml and 10ng/ml in relation to positive blood culture result. At cut-off value of 0.5ng/ml, the sensitivity, specificity, PPV, NPV, accuracy and likelihood ratio are 100%, 54.7%, 44.1%, 100.0%, 66.7% and 2.2 respectively, which implies that this cut off is not discriminatory for systemic bacterial infection due to high false positivity, low accuracy and low likelihood ratio. At cut-off value of 2ng/ml, the sensitivity, specificity, PPV, NPV, accuracy and likelihood ratio are 100.0%, 76.0%, 59.8%, 100%, 82.3%, and 4.2 respectively, which ind-

icates that this cut off is slightly more discriminatory for systemic bacterial infection due to lower false positivity, higher accuracy, and higher likelihood ratio. At a cut-off value of 10 ng/ml, the sensitivity, specificity, PPV, NPV, accuracy and likelihood ratio are 94.9%, 98.6%, 96.2%, 98.2%, 97.7% and 69.9 respectively, which indicate that this cut-off value is highly discriminatory for systemic bacterial infection due to very low false positivity (although few false negativity), very high accuracy and very high likelihood ratio (Table 4).

Association of PCT levels with blood cell count
 Univariate analysis of association of PCT levels with WCC showed that the odd of an abnormal white blood cell count (leukopaenia or leukocytosis) is lower with PCT value < 0.5 ng/ml (OR=0.047, 95% CI=0.025-0.087, $p<$ 0.0001) but at PCT value of ≥ 0.5 - < 2 ng/ml, abnormal WCC was not significantly associated with PCT value (OR=0.7451, 95% CI=0.3991-

1.391, $p=$0.4622), while the odd of abnormal WCC is higher with PCT value ≥ 2 - < 10ng/ml (OR=5.012, 95% CI=2.413-10.412, $p<$0.0001) and PCT value > 10ng/ml (OR=41.07, 95% CI =12.548 - 134.43, $p<$0.0001) (Table 5). This implies that the probability of abnormal WCC is higher when there is elevated serum PCT level.

Association of PCT levels with qSOFA score
 Univariate analysis of the association of serum PCT level with qSOFA cut off score of 2 showed that at PCT < 0.5ng/ml, the OR was 0.162 (95% CI=0.020-1.302, $p=$0.087), at PCT ≥0.5-<2ng/ml, the OR was 0.0515 (95% CI= 0.184 -1.638, $p=$0.283), at PCT ≥2-<10ng/ml, the OR was 0.603 (95% CI=0.203-1.791, $p=$ 0.362) and at PCT ≥ 10ng/ml, OR was 6.188 (95% CI=0.768-49.841, $p=$0.086) (Table 6). This analysis showed that there is no statistically significant association between PCT level and qSOFA cut off score of 2.

Table 4: Diagnostic parameters of PCT at different cut-off values compared with blood culture as "gold standard"

PCT levels (ng/ml)	Positive		Negative		Total (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Accuracy (%)	LR
	TP (%)	FP (%)	TN (%)	FN (%)							
0.5	79 (26.3)	100 (33.3)	121 (40.3)	0	300 (100)	100 (95.4 - 100)	54.8 (47.9 - 61.5)	44.1 (36.7-51.7)	100 (97.0-100)	66.7	2.2
2.0	79 (26.3)	53 (17.7)	168 (56.0)	0	300 (100)	100 (99.4 - 100)	76.0 (69.8 - 81.5)	59.8 (50.9-68.3)	100.0 (97.8-100)	82.3	4.2
10.0	75 (25.3)	3 (1.0)	218 (72.7)	4 (1.3)	300 (100)	94.9 (87.5 - 98.6)	98.6 (96.1 - 99.7)	96.2 (89.2-99.2)	98.2 (95.5-99.5)	97.7	69.9

PCT = Procalcitonin; TP=True Positive; FP=False Positive; TN=True Negative; FN=False Negative; PPV = Positive Predictive Value; NPV=Negative Predictive Value; LR = Likelihood Ratio; CI = Confidence Interval

Table 5: Univariate analysis of association of categories of serum PCT levels with abnormal white cell count

PCT values (ng/ml)	Abnormal WBC (%) (leucocytosis or leucopenia)	Normal WBC (%)	Total (%)	OR (95% CI)	p value
<0.5	18 (14.8)	103 (85.2)	121 (100)	0.047 (0.025 - 0.087)	<0.0001*
≥0.5 - <2	22 (46.8)	25 (53.2)	47 (100)	0.7451 (0.3991 - 1.391)	0.4622
≥2 - <10	44 (81.5)	10 (18.5)	54 (100)	5.012 (2.413 - 10.412)	<0.0001*
≥10	75 (96.2)	3(3.8)	78 (100)	41.07 (12.548 - 134.43)	<0.0001*
Total	**159 (53.0)**	**141 (47.0)**	**300 (100)**		

PCT= Procalcitonin, WBC= White blood cell, OR= Odd ratio, and CI= Confidence interval; * = statistically significant

Table 6: Univariate analysis of association of categories of PCT levels with qSOFA score

PCT value (ng/ml)	qSOFA score ≥2 (%)	qSOFA score <2 (%)	Total (%)	OR (95% CI)	p-value
<0.5	112 (92.6)	9 (7.4)	121 (100)	0.162 (0.020 - 1.302)	0.087
≥0.5 - <2	41 (87.2)	6 (12.8)	47 (100)	0.515 (0.184 - 1.638)	0.283
≥2 - <10	45 (83.3)	9 (16.7)	54 (100)	0.603 (0.203 - 1.791)	0.362
≥ 10	77 (98.7)	1 (1.3)	78 (100)	6.188 (0.768-49.841)	0.086
Total	**275 (91.7)**	**25 (8.3)**	**300 (100)**		

PCT= Procalcitonin, qSOFA quick Sequential Organ Failure Assessment, WBC= White blood cell, OR= Odd ratio, and CI= Confidence interval
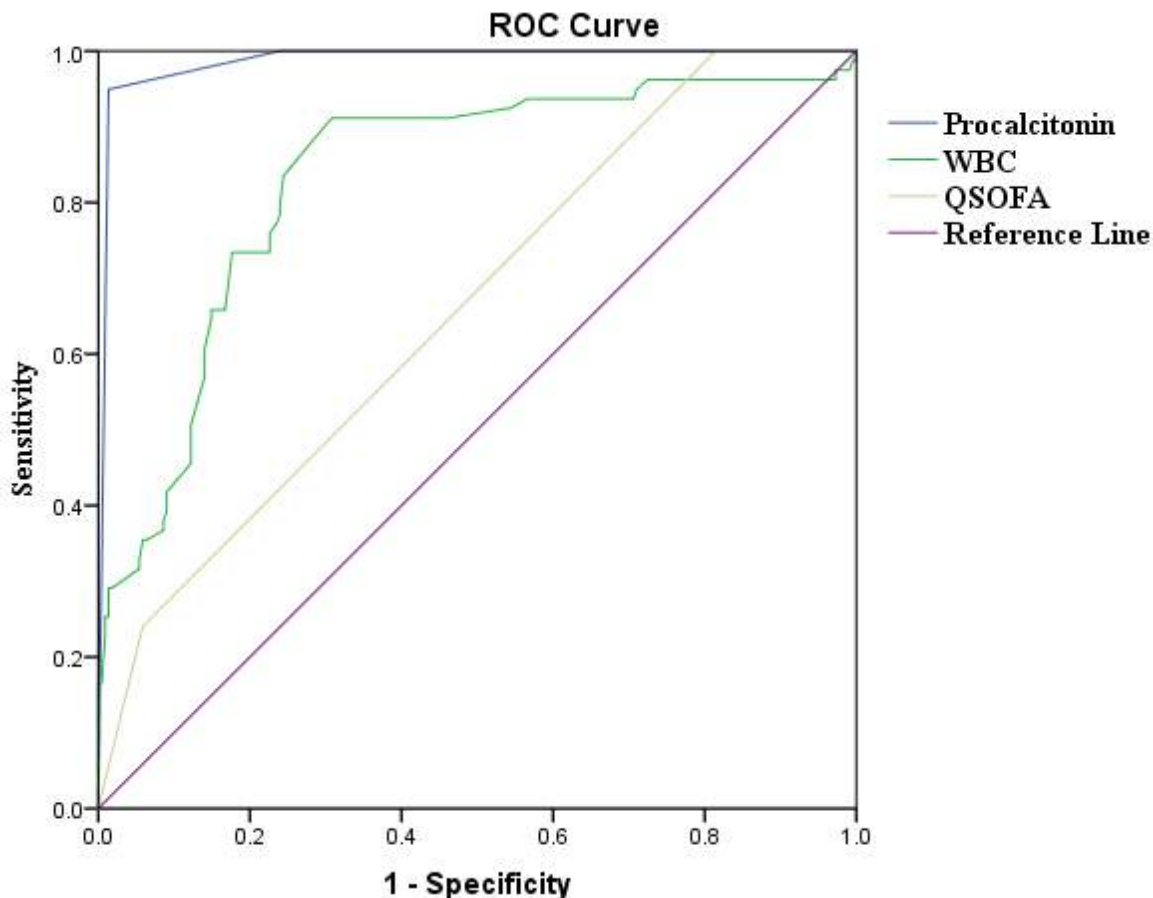
Parameters	AUC	95% CI	p-value
Procalcitonin	0.987	0.976-0.999	<0.001*
WBC	0.832	0.777-0.887	0.003*
qSOFA	0.661	0.594- 0.729	0.154

AUC=area under the curve; CI=confidence interval; *=statistically significant; qSOFA = quick sequential organ failure assessment; WBC=white blood cell; ROC=receiver operating characteristics

Fig 1: Comparison of diagnostic usefulness of PCT, WCC and qSOFA in sepsis using receiver operating characteristics curve analysis

Comparison of diagnostic values of PCT, WCC and qSOFA score in adult sepsis

The receiver operating characteristics (ROC) curve analysis was performed to determine the diagnostic usefulness of PCT compared to white cell count (WCC) and qSOFA score in the selected patients with systemic bacterial infections. PCT had higher area under the curve (AUC) of 0.987 (95% CI=0.976-0.999, *p*<0.0001) than that of WCC with AUC of 0.832 (95% CI=0.777-0.887, *p*=0.003) and qSOFA with AUC of 0.66 (95% CI = 0.594 -

0.729, *p*=0.154). Overall, PCT levels showed a higher diagnostic usefulness in patients with sepsis (*p*<0.001) and is a better tool to guide antibiotic therapy in patients with suspected sepsis when compared to WCC and qSOFA (Fig 1).

While PCT is a better tool, WCC (from statistical analysis) is equally useful as shown by AUC of 0.832 and *p*=0.003 (which is statistically significant). However, qSOFA appear not to be so useful in this study by low AUC of 0.666 and *p*=0.154 (which is not statistically significant). The ROC curve findings agree with the statistical analysis in the tables above.

Discussion:

High levels of serum PCT among adult patients with culture proven sepsis in this study have been reported in previous studies in Nigeria (13) and elsewhere (19,22). These studies reported serum PCT level as a sensitive and specific marker for detection of systemic bacterial infections. At PCT cut-off values of 0.5ng/ml, 2ng/ml and 10ng/ml, sensitivity, specificity, PPV, NPV, accuracy and likelihood ratio were reported relative to positive blood culture. At PCT cut off value of 0.5 ng/ml, the sensitivity and NPV are high (100%) but the specificity is reduced (54.8%) and PPV is much reduced (44.1%). This cut off value is therefore not discriminatory for systemic bacterial infection because of the high false positives and low positive likelihood ratio (PLR) of 2.2, but may confirm absence of systemic bacterial infection because the predictive value of a negative test (at PCT< 0.5ng/ml) is high. This is in agreement with the study of Liaudat et al., (23) who reported PCT as an early marker of sepsis in hospitalized patients and found a high NPV (95%) at PCT cut-off of 0.5ng/ml. Similarly, Bossink and colleagues (24) reported a 90% NPV at the same PCT cut-off value. Our finding is also consistent with previous reports on neonatal sepsis in Nigeria and South Africa (13, 25).

At PCT cut off value of 2 ng/ml, the sensitivity and NPV are high (100%) but the specificity, PPV and positive likelihood ratio (PLR) are a little higher (76.0%, 59.8%, and 4.2 respectively) than values reported at PCT cut off of 0.5 ng/ml. These values indicate that this cut off is slightly more discriminatory for systemic bacterial infections due to lower false positivity. At PCT cut off of 10ng/ml, the sensitivity, specificity, PPV, NPV, and accuracy are high (94.6%, 98.6%, 96.2%, 98.2% and 97.7% respectively), and the positive likelihood ratio is higher (69.9), which implies that this

cut off value is highly discriminatory for systemic bacterial infections due to the very low false positivity, very high accuracy and very high positive likelihood ratio. Therefore, patients with PCT level of ≥ 10ng/ml have very high probability of having systemic bacterial infections and physicians can objectively commence empirical antibiotic therapy while awaiting culture result. This finding agrees with the report of Arowosegbe et al., (13) in paediatrics population in Nigeria.

Univariate analysis of the association of PCT with abnormal WCC (leukocytosis or leukopenia) showed that the odd ratio of obtaining abnormal WCC progressively increased with increasing PCT levels, and at PCT level of ≥10 ng/ml, there was statistically significant association between elevated PCT level and abnormal WCC. This implies that patients with systemic bacterial infections who have elevated serum PCT level will most likely have abnormal WCC. This finding is consistent with the previous report of Magrini et al., (26). The receiver operating characteristics (ROC) curve analysis for PCT in predicting systemic bacterial infections showed area under the curve (AUC) of 0.987 (95% CI=0.976–0.999), which implies that PCT is a useful diagnostic tool for early diagnosis of systemic bacterial infection. This is in agreement with the study of Boraey and colleagues (27) in Egypt who reported AUC of 0.92 on ROC curve analysis. Ballot and colleagues (25) also reported a similar AUC of 0.778 in South Africa but previously, a lower AUC was reported in Nigeria (13).

From the comparison of the diagnostic values of PCT in detecting systemic bacterial infection with other screening parameters such as WCC and qSOFA using ROC curve analysis, the AUC was higher for PCT (0.987) than for WBC (0.832) and qSOFA (0.661), which is comparable to the reports of Magrini et al., (26), Park and colleagues (28), and Mueller et al., (5). Therefore, analysis of the results from this study revealed that PCT is a better tool, but WCC may equally be useful as shown by the AUC of 0.832 and p=0.003 (which is statistically significant), implying that WCC can serve as a useful alternative if PCT is not available, especially in low resource settings. However, qSOFA score appear not to be useful in this study as shown by the low AUC of 0.66 and *p*=0.154 (which is not statistically significant).

Blood culture is considered the "gold standard" for the laboratory evaluation of a patient with suspected systemic bacterial infections such as sepsis. In this study, 26.3% (79/300) of the blood cultures yielded positive

results for bacterial pathogens. The commonest bacterial isolate was *Staphylococcus aureus* (29.1%, 23/79), followed by *Klebsiella pneumoniae* (19.0%, 15/79) and *Escherichia coli* (16.5%, 13/79). This microbial pattern will enable physicians in making appropriate choice of antibiotics to be used in empirical therapy during initial management of patients with sepsis. The blood culture positivity rate and microbial pattern in this study are in keeping with the report of Kingsley and colleagues (29) in south-south Nigeria who reported 23.4% culture positivity although lower than 31.4% reported in southwest Nigeria (30).

We also noted that 3% of the blood cultures yielded skin contaminants, which may be attributed to the process of blood collection. Interestingly, serum PCT level in all the patients with contaminants was less than 0.5 ng/ml. This further supports the use of PCT levels as a tool to exclude contamination (when PCT level will be <0.5ng/ml) in addition to confirming severe bacterial infections (when PCT levels will be ≥10ng/ml), especially in low resource settings where a single blood culture is often used. This observation also corroborated a previous report that PCT levels can be used to differentiate true positive from false positive blood cultures (22).

Conclusion:

Most studies on diagnostic evaluation of PCT in this part of the world had focused on paediatric populations. Our findings support the usefulness of PCT as a biomarker for early diagnosis of sepsis in adult patients. This will also bring about timely intervention and appropriate antimicrobial use in managing critically ill adult patients, and because of its low turn-around time (<30mins), PCT estimation will help physicians more to rationally decide on antibiotic therapy, and a low PCT level may be used to rule out bacterial sepsis.

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Original Article   **Open Access**

Phytochemical study and evaluation of the antiviral activity of aqueous extracts of three medicinal plants; *Xylopia aethiopica*, *Gliricidia sepium* and *Ocimum gratissimum* used in Cote d'Ivoire

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Abstract:

Background: The present work is part of the exploration of new antiviral molecules to combat antimicrobial resistance. In purpose, this study determined the phytochemical analysis, cytotoxicity and antiviral activity of extracts from three Ivorian medicinal plants; *Gliricidia sepium, Ocimum gratissimum* and *Xylopia aethiopica* against poliovirus 1, a non-enveloped RNA virus.
Methodology: Aqueous extract of the three plants, which were identified at the herbarium of National Floristic Center Abidjan, was done using a previously described method. The precipitation or staining technique was used to highlight the chemical groups in the three extracts while the polyphenol content of each extract was assessed by the colorimetric method. Cytotoxicity and antiviral activity tests were performed in 96-well plates. Cytotoxicity of each extract on L20B (a genetically engineered mouse cell line) was determined by observation of the cell line carpet. Antiviral activity of three extracts against poliovirus type I was determined after 72 hours using an assay that measures inhibition of the cytopathic effect on cell culture.
Results: The three plant extracts contain polyterpenes, sterols and polyphenols, flavonoids, catechetical tannins, saponosides and quinones but none of the extract contains gallic tannins. With the exception of *O. gratissimum*, alkaloids were found in extracts from the two other plants, and extract of *G. sepium* was richer in polyphenol than the other two extracts. The cell carpet of L20B after 72 hours contact period with three extracts remained intact at concentrations ranging from 2 to 1000 µg/ml. The aqueous extract of *G. sepium* showed higher antiviral activity on poliovirus 1 (74.569%) at 2µg/ml than the extracts of *O. gratissimum* (45.6112%) and *X. aethiopica* (44.5247%) after 72 hours of incubation.
Conclusion: The extract of *G. sepium* showed potent antiviral activity against poliovirus 1 than that of *O. gratissimum* and *X. aethiopica*. This was justified by its higher polyphenol content than the two extracts.

Keywords: *Gliricidia sepium*, *Ocimum gratissimum*, *Xylopia aethiopica*, Phytochemistry, Cytotoxicity, Antiviral

Etude phytochimique et évaluation de l'activité antivirale d'extraits aqueux de trois plantes médicinales; *Xylopia aethiopica*, *Gliricidia sepium* et *Ocimum gratissimum* utilisés en Côte d'Ivoire

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Résumé:

Contexte: Le présent travail s'inscrit dans le cadre de l'exploration de nouvelles molécules antivirales pour lutter contre la résistance aux antimicrobiens. Dans le but, cette étude a déterminé l'analyse phytochimique, la cytotoxicité et l'activité antivirale d'extraits de trois plantes médicinales ivoiriennes; *Gliricidia sepium, Ocimum gratissimum* et *Xylopia aethiopica* contre le poliovirus 1, un virus à ARN non enveloppé.
Méthodologie: L'extrait aqueux des trois plantes, qui ont été identifiées à l'herbier du Centre National de Floristique d'Abidjan, a été réalisé selon une méthode précédemment décrite. La technique de précipitation ou de coloration a été utilisée pour mettre en évidence les groupements chimiques dans les trois extraits tandis que la teneur en polyphénols de chaque extrait a été évaluée par la méthode colorimétrique. Des tests de cytotoxicité et d'activité antivirale ont été réalisés dans des plaques 96 puits. La cytotoxicité de chaque extrait sur L20B (une lignée cellulaire de souris génétiquement modifiée) a été déterminée par l'observation du tapis de la lignée cellulaire. L'activité antivirale de trois extraits contre le poliovirus de type I a été déterminée après 72 heures en utilisant un test qui mesure l'inhibition de l'effet cytopathique sur la culture cellulaire.
Résultats: Les trois extraits végétaux contiennent des polyterpènes, des stérols et des polyphénols, des flavonoïdes, des tanins catéchétiques, des saponosides et des quinones mais aucun extrait ne contient de tanins galliques. A l'exception d'*O. gratissimum*, des alcaloïdes ont été trouvés dans les extraits des deux autres plantes, et l'extrait de *G. sepium* était plus riche en polyphénols que les deux autres extraits. Le tapis cellulaire de L20B après 72 heures de contact avec trois extraits est resté intact à des concentrations allant de 2 à 1000 µg/ml. L'extrait aqueux de *G. sepium* a montré une activité antivirale plus élevée sur le poliovirus 1 (74,569%) à 2 µg/ml que les extraits d'*O. gratissimum* (45,6112%) et de *X. aethiopica* (44,5247%) après 72 heures d'incubation.
Conclusion: L'extrait de *G. sepium* a montré une activité antivirale puissante contre le poliovirus 1 que celle de *O. gratissimum* et *X. aethiopica*. Ceci était justifié par sa teneur en polyphénols plus élevée que les deux extraits.

Mots clés: *Gliricidia sepium*, *Ocimum gratissimum*, *Xylopia aethiopica*, Phytochimie, Cytotoxicité, Antivirale

Introduction:

About fifty years after scientific research began on antiviral agents, viral diseases remain a major global concern. This is due on the one hand to the toxicity of the many drugs developed (1,2) and on the other hand to the rapid appearance of drug-resistant strains (3,4,5). In order to discover new effective antiviral molecules, alternative avenue of investigation could be exploited, which is medicinal plants used for health care practices in low-income countries (6). Indeed, compounds isolated from medicinal plants such as flavonoids, tannins, proteins, polysaccharides, and alkaloids have been reported to exhibit antiviral activity (7). A report indicates that a flavone (natural flavonoid) isolated from *Agastache folium* has good antiviral activity in cell culture against most picornaviruses without showing toxicity to growing cells (8).

An excellent model for studying viral replication is the poliovirus, an RNA virus, belonging to the Picornaviridae family. The latter is responsible for poliomyelitis, a disease eradicated by many countries but which remains a threat to the whole world. Researchers (9) have reported that pathologies such as meningitis, myocarditis, encephalitis and respiratory diseases can be caused by enteroviruses, the genus to which poliovirus belongs.

Gliricidia sepium, O. gratissimum, and *X. aethiopica* are three medicinal plants used against viral diseases on the basis of an ethno-botanical survey carried out in the district of Abidjan, Côte d'Ivoire (10). *G. sepium* has been reported to have antibacterial, antifungal and antiviral properties (11). *Ocimum gratissimum* is used in ethnomedicine generally for the treatment of the upper respiratory tract (cough, pneumonia, etc.) and digestive disorders (diarrhea, dysentery, etc.), skin pathologies, fever, headaches and conjunctivitis (12,13). The extracts obtained by decoction of the dried fruits of *X. aethiopica* are used to treat various respiratory, digestive and inflammatory diseases and infections, including dysentery and malaria (14).

The general objective of this study is to investigate the phytochemical constituents and evaluate the antiviral activity of aqueous extracts of these three Ivorian medicinal plants; *G. sepium*, *O. gratissimum*, and *X. aethiopica.* This specifically will involve carrying out on the one hand, qualitative phytochemical analysis and dosage of polyphenols of the extracts, and on the other hand, the cytotoxicity and antiviral activity of the extracts respectively on L20B cells (a genetically engineered mouse cell line) and the poliovirus type 1.

Materials and method:

Plant materials

The plant material consists of the leaves of *G. sepium*, *O. gratissimum* and fruits of *X. aethiopica*. The leaves of *G. sepium* and

Antiviral activity of three Ivorian medicinal plants

Afr. J. Clin. Exper. Microbiol. 2022; 23 (2): 141-148

O. gratissimum were collected in August 2021 in the district of Abidjan while the fruits of *X. aethiopica* were bought at the Adjamé market. The three plants were identified at the National Floristic Center under the respective herbarium numbers; UCJ010419, UCJ008879 and UCJ001462.

Preparation of aqueous extracts

The different leaves were cleaned and dried in a dry place, ventilated away from light for five days except for the fruits which were dry. The preparation of the total aqueous extracts was carried out according to the method of Zirihi et al., (15). One hundred grams (100g) of vegetable powder was dissolved in 2 liters of distilled water. The mixture was then homogenized 10 times at a rate of 2 minutes per revolution using a mixer (Blender Bruon H-999A).

The homogenate obtained was drained in a square of cloth and then filtered successively three times on cotton wool and then on Wattman paper (3 mm). The filtrate was evaporated at 55°C using a Venticell® type oven for three days (72 h) to obtain a powder which is the total aqueous extract. At the end of the extractions, R = (100 x m)/M, where 'R' is yield (%), 'm' is mass of dry extract (g), and 'M' is mass of plant material used (g).

Phytochemical study: qualitative analysis and polyphenol content

The precipitation or staining technique was used to highlight the desired chemical groups of alkaloids, tannins, polyphenols, flavonoids, quinones, saponosides, sterols and polyterpenes in aqueous extracts of *O. gratissimum*, *X. aethiopica* and *G. sepium* (6,16,17, 18).

The polyphenol content of each extract was evaluated according to the Folin-Ciocalteu colorimetric method described by Bakchiche and Gherib (19) with some modifications. To 1 ml of each plant extract are added respectively 2.5 ml of Folin-Ciocalteu diluted 1/10 in distilled water and after 6 min, 2 ml of sodium carbonate (20%). The whole is incubated at room temperature and protected from light for 2 hours. Optical densities (OD) were read with a spectrophotometer at 730 nm against blank prepared in the same manner, replacing the extract with distilled water. The gallic acid which constitutes our standard was prepared under the same conditions as the extract with a mixture of ethanol/water solvent (50:50, V/V) at concentrations ranging from C1 to C6 tubes.

L20B cells and poliovirus 1

L20B cells (genetically modified mouse cell lines) were cultured in Eagle's minimal essential medium (MEM) (Mediatech Cellgro, VA) supplemented with 10% fetal bovine serum, PBS, penicillin (100 IU) and streptomycin (100 mg/mL) (Mediatech Cellgro®). The latter were maintained in a humidified atmosphere at 36° C in 5% CO_2. Sabin I poliovirus [VP1, CDC, Atlanta], non-enveloped RNA virus was provided by the Department of Epidemic Viruses of the Institut Pasteur in Côte d'Ivoire. The viral suspension (contained in a 2 ml cryotube) was frozen at -80°C before use.

Determination of the cellular toxicity of extracts on L20B cells

The cytotoxicity test was carried out according to the method described by Ojo et al., (20) with some modifications. The extracts of *G. sepium*, *O. gratissimum* and *X. aethiopica* dissolved in 2% MEM (minimum essential medium) were distributed in 10 microtubes each. The concentrations vary respectively from 1 mg/ml to 2 µg/ml (cascade dilution). The extracts at different concentrations were placed in 96-well microplates. Each well contains 100 µl of extract and 100 µl of L20B cell suspension (0.25×10^6 cells/ml). The controls are of two types: a negative control consisting of cells without extract and a positive control consisting of cells and Tritton. Each microplate covered with an adhesive film is incubated for 3 days at 36°C in 5% CO_2. Cell morphology (e. g. rounding, narrowing detachment) is examined by light microscopy after 24, 48 and 72 hours of incubation.

Antiviral activity

The evaluation of the antiviral activity was carried out by the method described by Meyer et al., (1996) with modifications. 50 µl of L20B cell suspension (0.25×10^6 cells/ml) were incubated with the suspensions of poliovirus I in equal proportion (1:1) at 37°C for 1 hour 30 min in a 96-well plate. 50 µl of the smallest non-toxic concentration of each extract (2 µg/ml) were added (in triplicate for each extract). Two types of control, a negative control (consisting of cell suspension without extract or virus) and a positive control (consisting of cell suspension and virus) were used.

The effect of each extract on poliovirus I was evaluated by determining the optical density (OD) at 490 nm in a Multiskan FC reader as; Percentage (%) CPE inhibition = (At-Ap)/(An-Ap) x 100, where 'An' is the average absorption of negative control (cell suspension without virus or extract), 'Ap' is the average absorption of the positive control (cell suspension with virus without extract), and 'At' is the average absorption of the sample tested.

Statistical analysis of data

The experiments were performed in triplicate and the data were analyzed using ANOVA. The value of the significance threshold

was 0.05. Statistical difference with a probability value less than 0.05 ($p<0.05$) was considered significant.

Results:

Extract yield

The yields obtained at the end of the extraction were respectively 13.01% for the leaves of *G. sepium*; 10.61% for the leaves of *O. gratissimum* and 9.81% for the fruits of *X. aethiopica*.

Phytochemical study

All the plant extracts contain polyterpenes, sterols and polyphenols, flavonoids, catechetical tannins, saponosides and quin-

ones. On the other hand, no extract contains gallic tannins. With the exception of *O. gratissimum*, alkaloids were found in extracts from other plants (Table 1).

The polyphenol contents of the plant extracts were determined from the calibration line for gallic acid (Y=5.1316x) (Fig 1). The polyphenol contents were reported in mg of gallic acid equivalent/g of dry extract (mg EAG/g ES). The *G. sepium* extract (0.13± 0.02 mg EAG/g ES) was richer in polyphenols while the extract of *O. gratissimum* and *X. aethiopica* showed low values for the same solvent used. These contents were respectively 0.08 ±0.003 mg EAG/g ES and 0.05±0.01 mg EAG/g ES (Fig 2).

Table 1: Phytochemical compounds in three Ivorian medicinal plants

Excerpts	Sterols and Poly-terpenes	Polyphenols	Flavonoids	Tannins		Quinones	Alkaloids		Saponins
				Cat.	Gal.		B	D	
G. sepium	+	+	+	+	-	+	+	+	+
O. gratissimum	+	+	+	+	-	+	-	-	+
X. aethiopica	+	+	+	+	-	+	+	+	+

Cat = Catechic, Gal = Gallic, B - Bouchardat, D = Dragendorff, + = Presence of the metabolite, **-** = Absence of the metabolite.
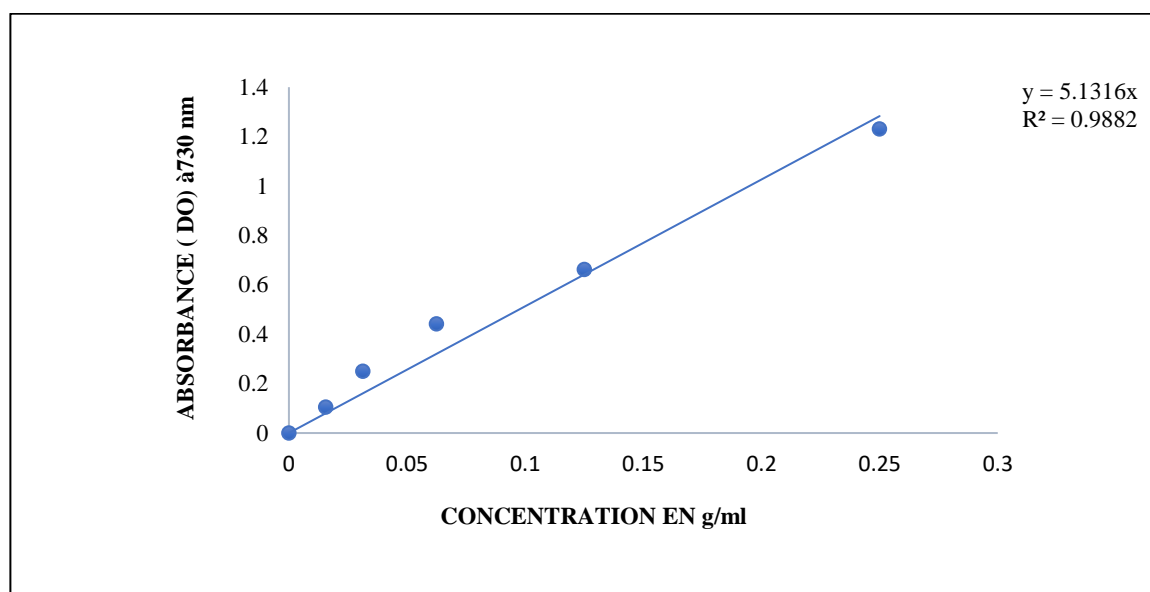
Fig 1: Gallic acid calibration curve
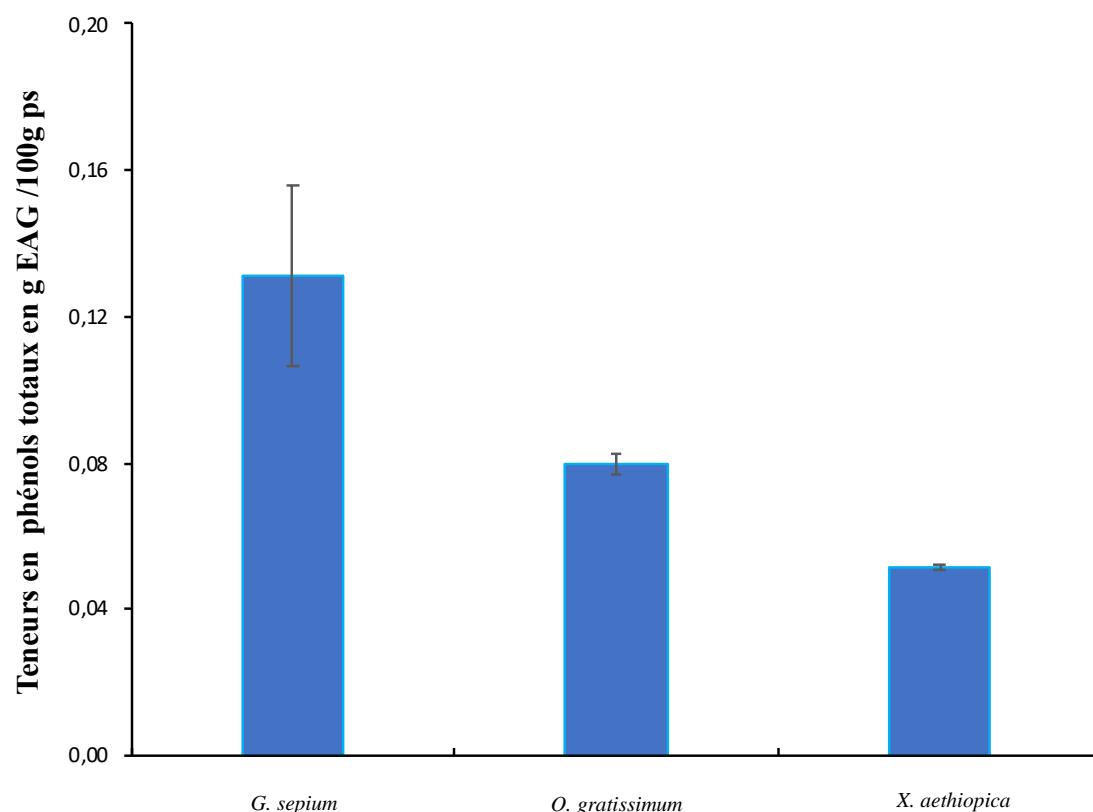
Fig 2: Polyphenol content of aqueous leaf extracts of *Gliricidia sepium*, *Ocimum gratissimum* and fruits of *Xylopia aethiopica*.

Cytotoxicity to L20B cell lines

An intact cell layer was observed after 72 hours of contact of the L20B cells with each extract at concentrations ranging from 2 (1.953125) to 1000 µg/ml (Table 2). The lowest concentration which had no cytotoxic effect on the L20B cells was 1.953125 µg/ml (~ 2 µg/ml), which was used to evaluate the antiviral activity of the three plant extracts.

Antiviral activity

The antiviral activity of the extracts of the three plants on poliovirus 1, evaluated by the percentage inhibition of the effect of the cytopathic effect, is shown in Table 3. The aqueous extract of *G. sepium* showed significantly higher antiviral activity (74.569%) than the extracts of *O. gratissimum* and *X. aethiopica* ($p<0.01$). The latter two extracts had statistically identical antiviral activity (45.6112 and 44.5247% respectively) on poliovirus 1 (Fig 3).

Discussion:

The experiments carried out at the phytochemical level made it possible to identify

Table 2: Effect of crude extracts on L20B cells

Extraits	Cellules	CONCENTRATION (µg/ml)										Contrôle négatif	Contrôle positif
		1,953125	3,90625	7,8125	15,625	31,25	62,5	125	250	500	1000		
G. sepium	L20B	-	-	-	-	-	-	-	-	-	-	-	+
O. gratissimum													
X. aethiopica													

(-): absence of toxicity, (+): presence of toxicity.

Table 3: Inhibition of the PCE of poliovirus 1 by the three plant extracts (at a concentration of 2 µg/ml)

Extraits	% moyen inhibition ± Ecart-type		Paramètre statistique d'ANOVA		
			dl	F	P
G. Sepium	74,569 ±	0,6096[a]	2	122,5	<0,01
O. gratissimum	45,6112 ±	3,1324[b]			
X. aethiopica	44,5247 ±	2,0099[b]			
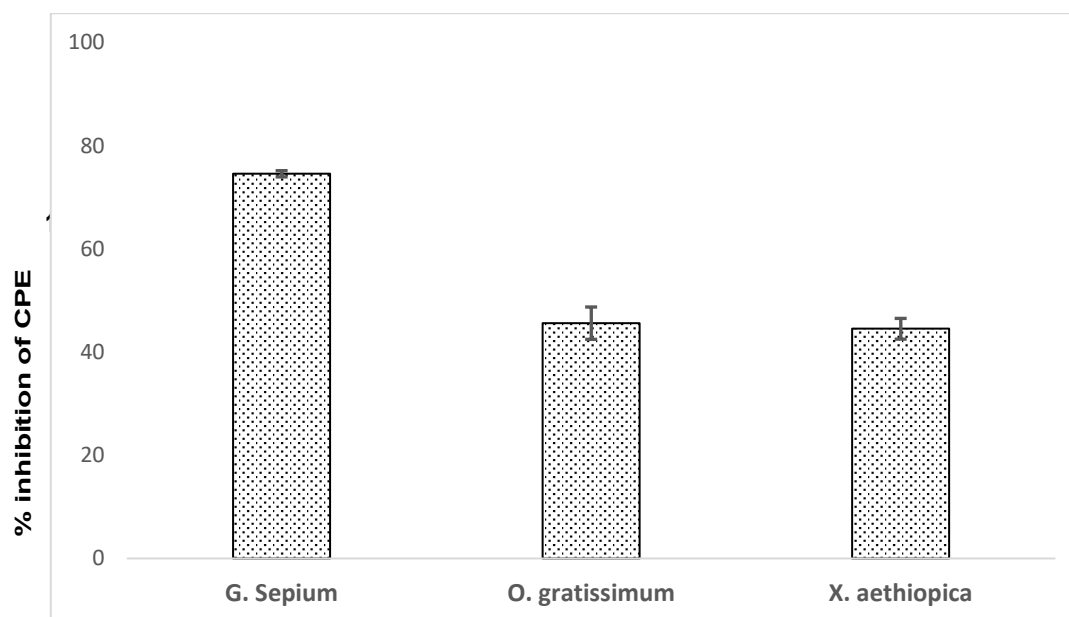
Fig 3: Inhibition of the PCE of poliovirus 1 in the presence of aqueous extracts of *G. sepium*, *O. gratissimum* and *X. aethiopica*

several secondary metabolites present in the extracts of the leaves of *G. sepium, O. gratissimum* and the fruit of *X. aethiopica*. The aqueous extraction yields revealed that *G. sepium* has a higher yield (13.01%) followed by *O. Gratissimum* (10.61%) and *X. aethiopica* (9.81%). This means that *G. sepium* could be richer in water-extractable metabolites because a link exist between extraction yields and biological activities (21).

In addition, the three extracts qualitatively contain polyphenols, flavonoids, catechetical tannins, saponins, quinones, sterols and polyterpenes. However, the lack of gallic tannins in the three extracts and alkaloids in the extract of *O. gratissimum* could indicate a lack of affinity with the extraction solvent. Recent work has indicated the presence of polyphenols, tannins and flavonoids in the aqueous extract of leaves of *O. gratissimum* (22), and the works of Okwu and Omodamiro (23) revealed the presence of tannins, alkaloids, flavonoids, saponins and polyphenols in the fruits of *X. aethiopica.* The polyphenol content of the *G. sepium* extract (0.13±0.02

mg EAG/g ES) was significantly higher than that of *O. gratissimum* and *X. aethiopica* (0.08 ±0.003 mg EAG/g ES and 0.05±0.01 mg EAG/g ES, respectively). Some researchers have reported that polyphenols are endowed with antioxidant capacity (24), but in addition, polyphenols, in particular flavonoids, are endowed with antiviral properties (25).

No cytotoxicity of the three plant extracts (at concentrations ranging from 2 µg/ml to 1 mg/ml) was seen on L20B cell (a genetically modified mouse cell line). Indeed, researchers have considered as active a plant extract which antiviral activity is detectable in at least two subsequent dilutions of the maximum non-toxic concentration in order to ensure that the activity is not directly correlated with the toxicity of the extract (26). The antiviral efficacy of the extract of *G. sepium* (at a concentration of 2 µg/ml resulted in a rate of inhibition of 74% of the cytopathic effect. As non-enveloped viruses have indeed been reported in the literature to exhibit high intrinsic resistance because of their structure (27), this result indicates a promising activity

of the extract of *G. sepium* in the development of plant antivirals.

Our study constitutes an avenue of investigation into the use of these types of antiviral drugs in the face of the phenomenon of microbial resistance to antimicrobial agents. In addition, previous study has revealed that polyphenols derived from plants, particularly flavonoids, act effectively on various viruses such as poliovirus, respiratory syncytial virus (RSV) and herpes simplex virus (HSV) (28). However, further study is needed to elucidate the mechanism of action of *G. sepium* extract. The extracts of *O. gratissimum* and *X. aethiopica* for their part inhibited the cytopathic effect of poliovirus 1 by 45.6112 and 44.5247% respectively. These levels, although being moderate, constitute an avenue for further exploration study of these two extracts plants on viruses.

Conclusion:

The antiviral activity of three medicinal plants selected from the Ivorian flora against poliovirus 1 was reported in the present study. The extract of *G. sepium* showed potent antiviral activity against poliovirus 1 than that of *O. gratissimum* and *X. aethiopica*. This was justified by its higher polyphenol content than the two extracts. Further research is needed to elucidate their mechanism of action of the extract by *G. sepium*. To our knowledge, this is the first report on the antiviral activity of *G. sepium*, *O. gratissimum* and *X. aethiopica* following the ethnobotanical survey carried out on the plants.

Conflicts of interests:

No conflict of interest is declared.

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Original Article　　　　　　　　　　　　　　　Open Access

Prevalence and risk factors for extended-spectrum β-lactamase-producing Gram-negative bacterial infections in hospitalized patients at a tertiary care hospital, southwest Nigeria

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Abstract:

Background: Clinical infections caused by extended-spectrum β-lactamase (ESBL)-producing bacteria constitute great burden to healthcare delivery with these resistant pathogens contributing largely to the magnitude and spread of antimicrobial resistance globally. Hence, knowledge of the risk factors for acquisition of infection caused by ESBL-producing bacteria is crucial to instituting prompt and appropriate treatment as well as prevention and control measures**.** This study investigated the risk factors associated with the prevalence of ESBL-producing Gram-negative bacteria (GNB) infections among hospitalized patients in Uniosun Teaching Hospital (UTH), Osogbo, Nigeria.
Methodology: A total of 359 hospitalized patients with clinical infections from whose clinical samples we isolated non-duplicate GNB were consecutively recruited. GNB were isolated following aerobic cultures of appropriate clinical samples and Microbact[TM]GNB 24E kit was used for species identification. All isolates were screened for ESBL production by the combination disc method. Relevant clinical and demographic information was obtained using a designed data collection form, and multivariate logistic regression analysis was used to identify associated risk factors.
Results: Ninety-four (26.2%) of the 359 patients had ESBL-producing GNB isolated from their clinical samples, with a preponderance of *Escherichia coli* (26.6%, n=25/94), although the most frequent ESBL-producer was *Stenotrophomonas maltophilia* (100%, n=2/2) and least frequent was *Pseudomonas aeruginosa* (2.6%, n=1/39). The study indicated that male gender, age group >60 years and farming were socio-demographic factors associated with significantly higher prevalence of ESBL-producing GNB infection. Other independent risk factors significantly associated with high prevalence of ESBL GNB infections were; (i) admission into intensive care unit and male surgical ward, (ii) presence of invasive devices such as intravenous line, endotracheal tube and urinary catheter, (iii) underlying conditions such as diabetes mellitus and benign prostatic hyperplasia, and (iv) immunocompromised state.
Conclusion: The information obtained from this study can serve as baseline data for designing strategy to prevent drug-resistant infections and transmission in our hospital.

Keywords*:* Prevalence, risk factors, extended-spectrum β-lactamase, Gram-negative bacilli

Facteurs de prévalence et de risque pour les infections de bactéries gram-négatives de la β-lactamase prolongées de la β-lactamase chez les patients hospitalisés dans un hôpital de soins tertiaires, au sud-ouest du Nigéria

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Résumé:

Contexte: Les infections cliniques causées par des bactéries de la β-lactamase de spectre prolongée (ESBL) constituent une grande charge à la livraison des soins de santé avec ces agents pathogènes résistants contribuant en grande partie à la magnitude et à la propagation de la résistance antimicrobienne mondiale. Par conséquent, la connaissance des facteurs de risque d'acquisition d'une infection causée par les bactéries produisant des ESBL est essentielle à l'institution de traitement rapide et approprié, ainsi que des mesures de prévention et de contrôle. Cette étude a enquêté sur les facteurs de risque associés à la prévalence des bactéries gram-négatives de l'ESBL (GNB) parmi les patients hospitalisés dans l'hôpital d'enseignement Uniosun (Uth), Osogbo, Nigéria.

Méthodologie: Un total de 359 patients hospitalisés avec des infections cliniques de laquelle les échantillons cliniques de laquelle nous avons isolé le GNB non dupliqué ont été recrutés consécutivement. GNB ont été isolés à la suite de cultures aérobies d'échantillons cliniques appropriés et de kit MicroBact[TM] GNB 24E a été utilisé pour l'identification des espèces. Tous les isolats ont été criblés pour la production ESBL par la méthode des disques combinées. Des informations cliniques et démographiques pertinentes ont été obtenues à l'aide d'un formulaire de collecte de données conçu et une analyse de régression logistique multivariée a été utilisée pour identifier les facteurs de risque associés.

Résultats: Quatre-vingt-quatorze (26,2%) des 359 patients avaient des GNB producteurs de BLSE isolés de leurs échantillons cliniques, avec une prépondérance d'*Escherichia coli* (26,6%, n=25/94), bien que le producteur de BLSE le plus fréquent soit *Stenotrophomonas maltophilia* (100.0%, n=2/2) et la moins fréquente était *Pseudomonas aeruginosa* (2,6%, n=1/39). L'étude a indiqué que le sexe masculin, le groupe d'âge > 60 ans et l'agriculture étaient des facteurs sociodémographiques associés à une prévalence significativement plus élevée d'infections à GNB productrices de BLSE. D'autres facteurs de risque indépendants significativement associés à une prévalence élevée d'infections à BLSE GNB étaient; (i) admission en unité de soins intensifs et en salle de chirurgie pour hommes, (ii) présence de dispositifs invasifs tels qu'une ligne intraveineuse, un tube endotrachéal et un cathéter urinaire, (iii) conditions sous-jacentes telles que le diabète sucré et l'hyperplasie bénigne de la prostate, et (iv) immunodéprimé Etat.

Conclusion: les informations obtenues à partir de cette étude peuvent servir de données de base pour la conception de la stratégie visant à prévenir les infections et la transmission résistantes à la drogue dans notre hôpital.

Mots-clés: Prévalence, facteurs de risque, β-lactamase de spectre prolongé, bacille Gram-négatif

Introduction:

Extended-spectrum β-lactamases (ESBL) are enzymes that confer on many Gram-negative bacilli (GNB) of the family Enterobacteriaceae the ability to hydrolyze β-lactam ring, thereby inactivating β-lactam antibiotics such as penicillins and cephalosporins including oxyimino-cephalosporins and monobactams which are common agents for treatment of clinical infections (1). ESBL-producing bacteria are particularly more worrisome especially in healthcare settings because of their high adaptability and efficient dissemination. The rising incidence of ESBL-producing GNB infections has raised serious health concerns globally. Prevalence of infections by ESBL-producers varies considerably and is largely dependent on local epidemiology as well as prevailing antimicrobial prescribing policies and patterns. Previous reports have shown the value to vary from 0-80% across different African regions which is essentially due to differences in levels of antibiotic use (2-5).

Increasing occurrence of clinical infections caused by ESBL-producing bacteria reduces therapeutic options for patients, and this situation has been made worse by inherent problems militating against medical care in the low-and-middle-income-countries (LMICs) including poor drug supply chain, ineffective health insurance, financial burden of out-of-pocket (OOP) drug procurement by patients, rudimentary laboratory diagnostics and under-developed hospital infection prevention and control. Infections by these "super bugs" have increased the usage of the few available 'last resort' reserved antibiotics like the carbapenems, leading to increasing resistance to these agents, with associated huge mortality.

Factors predisposing patients to infections by ESBL-producing pathogens are numerous, diverse and commonly related to infection sites and interventions. Recent hospitalization and prolonged exposure to antibiotics were among the most commonly documented risks for acquisition of clinical infections caused by ESBL-producing bacteria (6-10). A systematic review of 51 studies showed that previous hospitalization and prolonged use of cephalosporins are the independent risk factors for infections by hospital-acquired ESBL-producing Entero bacteriaceae among hospitalised paediatrics patients (11). Utilization of invasive devices is another risk for infections by the drug-resistant pathogens (12). Furthermore, it has also been

widely reported that chronic and immuno-suppressive illnesses including diabetes mellitus (DM) and malignancies are predisposing factors harbouring infection by ESBL-producing bacteria (7, 10, 11).

Despite the increasing prevalence of ESBL-producing bacterial infections locally and globally with many reports on the epidemiology of these infections, risk factors for the acquisition of such infections have not been well defined in our hospital settings. Early identification of patients at risk of infection with ESBL-producing bacteria will enhance prescribing of most effective empirical therapy and applying appropriate infection prevention and control (IPC) measures to limit the spread of these multidrug-resistant infections. This can reduce the complications associated with ESBL infections, cost of treatment and emergence of antibiotic resistance, and improve patients' survival. In this study, we investigated the risk factors for ESBL-GNB infections from a previous study that determined the prevalence and molecular characteristics of ESBL-producing GNB for a more proactive approach in patient case management and effective IPC in our hospital.

Materials and method:

Study setting

This study was conducted between January and December 2016 at the Ladoke Akintola university of Technology (LAUTECH) Teaching Hospital, now Uniosun Teaching Hospital (UTH), Osogbo, Nigeria. UTH is a 600-bed hospital which provides healthcare services in various specialties for the people of Osogbo and its environs.

Ethical approval

Ethical approval for this study was obtained from Ethics Committee of LAUTECH Teaching Hospital, Osogbo, Nigeria (Protocol Number- LTH/REC/2015/06/05/210).

Study design and sampling method

This was a descriptive cross-sectional study in which 359 hospitalized patients with symptoms and signs of clinical infections were consecutively recruited over a period of one year. Consecutive non-duplicate Gram-negative bacilli (GNB) were isolated from the patients' clinical samples at the diagnostic microbiology laboratory of the hospital. Clinical and demographic information were obtained from patients' clinical folders into a designed data collection form.

Collection of samples and laboratory analysis

All urine, faecal, blood, cerebrospinal fluids, sputum, wound and aspirates samples were collected by managing physicians and surgeons from in-patients with clinical symptoms and signs of infection. The samples were processed using standard methods by inoculating all the clinical samples except stool on Blood and MacConkey agar (Oxoid, Basingstoke, United Kingdom). Stool samples were cultured on Deoxycholate Citrate agar (Oxoid, Basingstoke, United Kingdom).

Identification of Gram-negative bacilli

Isolates were identified by colonial morphology, Gram staining and standard biochemical tests including the use of Microbact™ GNB 24E (Oxoid, Basingstoke, United Kingdom) which is a standardised microsubstrate system for the identification of Enterobacteriaceae and miscellaneous GNB based on colour changes in the test due to pH change and/or substrate utilization. The test results generated an octal code which was entered into the Microbact computer aided identification package to give an identification profile for the organism.

Antimicrobial susceptibility test

All Gram-negative bacilli were tested against gentamicin (10µg), amoxicillin-clavulanate (20/10µg), amikacin (10µg), meropenem (10µg), ceftazidime (30µg), cefotaxime (30µg), ciprofloxacin (5µg), cefepime (30µg), ceftriaxone (30µg), cefoxitin (30µg), cotrimoxazole (1.25/23.75µg), and piperacillin-tazo-bactam (100/10µg) (Oxoid, England, UK) using the Kirby-Bauer disc diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (13). Zones of inhibition diameters were measured and interpreted using the guidelines. Isolates which were not sensitive to one or more of the tested third generation cephalosporins and/or aztreonam were regarded to be screening positive for ESBL-production and were subjected to the confirmatory testing.

Phenotypic detection of extended spectrum beta-lactamase (ESBL)

Phenotypic confirmatory test for ESBL production was carried out by combination disc method on GNB that showed resistance to one or more of the tested third generation cephalosporins. This test utilised both single discs of cefotaxime and ceftazidime and their respective clavulanic acid-augmented (13, 14). An increase of 5 mm or more in zones of inhibition of combination discs (cephalosporins with clavulanate) when compared with their respective single discs was taken as phenotypic confirmatory evidence of ESBL production. *Klebsiella pneumoniae* ATCC 700 603 and *Escherichia coli* ATCC 25922 respectively served as positive and negative controls.

Data analysis

The R statistical software package (version 3.3.0) was used for data analysis (15). Univariate analysis was performed to determine the variables associated with acquisition of ESBL-producing GNB. Continuous and categorical variables were compared using Wilcoxon rank-sum and Fisher exact tests respectively, and statistical testing performed using 2-tailed tests. Multivariate logistic regression was used to measure the association between ESBL infection and the prior identified risk factors adjusting for potential confounding variables, with all variables associated with acquisition of ESBLs in the bivariate analysis were included in the initial (full) model. Adjusted odds ratio (OR) and 95% confidence intervals were calculated on the basis of the final multivariate regression model.

Results:

A total of 359 consecutively recruited hospitalized patients from whom GNB were isolated from their different clinical samples over a period of one year (January–December 2016) were studied. One hundred and sixty-eight (46.8%) were males while 191 (53.2%) were females, with a male to female ratio of 0.88 (Table 1). The age range of the patients is 5 days to 83 years while the mean age is 38.9 ± 21.16 years. Patient age > 60 years were the most frequent (n=72; 20.1%) followed by those within age range 51-60 years (n=66; 18.4%) and 31-40 years (n= 58; 16.4%). The patients were predominantly traders (n=83; 23.1%), artisans (n= 54; 15%), students (n=51; 14.2%) and civil servants (n=40; 11.1%).

Table 1: Socio-demographic characteristics of hospitalized patients at Uniosun Teaching Hospital, Osogbo (Jan – Dec, 2016)

Variables	Frequency	Percentage
Age group (years)		
≤10	54	15.0
11-20	33	9.2
21-30	24	6.7
31-40	59	16.4
41-50	51	14.2
51-60	66	18.4
>60	72	20.1
Mean age (± SD)	38.91±21.16	
Gender		
Male	168	46.8
Female	191	53.2
Occupation		
Artisan	54	15.0
Civil Servant	40	11.1
Clergy	4	1.1
Farming	20	5.6
Professional	12	3.3
Retiree	27	7.5
Student	51	14.2
Teaching	4	1.1
Trading	83	23.1
Unemployed	34	9.5
Unspecified	30	8.4
Ward/Clinic		
Female Medical Ward	67	18.7
Male Medical Ward	66	18.4
Male Surgical Ward	44	12.3
Gynaecology	24	6.7
Female Surgical Ward	21	5.8
Neonatal ward	19	5.3
Children ward	18	5.0
Children emergency unit	17	4.7
Antenatal ward	16	4.5
Intensive care unit	15	4.2
Accident and Emergency	12	3.3
Orthopaedics ward	11	3.1
Postnatal ward	9	2.5
Burns	8	2.2
Renal	7	1.9
Psychiatric	4	1.1
Ear, nose and throat ward	1	0.3

Table 2: Clinical characteristics of hospitalized patients at Uniosun Teaching Hospital, Osogbo, Nigeria (Jan – Dec 2016)

Clinical characteristics	Number	Percentage
Clinical Diagnoses		
Urinary tract infection	146	40.7
Sepsis	72	20.1
Chronic wound infection	55	15.3
Surgical site infection	32	8.9
Pneumonia	28	7.8
Meningitis	10	2.8
Gastroenteritis	7	1.9
Chronic osteomyelitis	5	1.4
Ear infection	2	0.6
Pyomyositis	2	0.6
Invasive devices		
IVL only	282	78.6
IVL, urocatheter	43	12.0
IVL, endotracheal tube, urocatheter	9	2.5
IVL, urocatheter, wound drains	4	1.1
IVL, abdominal drains	3	0.8
IVL, endotracheal tube	2	0.6
Urocatheter only	2	0.6
None	14	3.9
Co-morbidities		
Hypertension	46	12.8
Malaria	38	10.6
Inguino-scrotal hernia	34	9.5
Diabetes	17	4.7
Head injury	5	1.4
Mania/Psychosis/Schizophrenia	4	1.1
Vaso-occlusive crisis	4	1.1
Malignancy	3	0.8
RTA/Fracture of bones	3	0.8
Ectopic Pregnancy	2	0.6
Cerebrovascular disease	2	0.6
None	201	56.0
Length of admission		
Less than 1 month	332	92.5
More than 1 month	25	7.0
Not available	2	0.6
Length of admission (days) Mean ± SD	11.8 ± 9.1	
Previous admission in the last 1 year	105	29.2
Previous ESBL culture	12	3.3
Previous antibiotic use in the last 3 months	93	25.9
Immuno-compromised state	85	23.7

IVL = Intravenous line; SD = Standard deviation; RTA = Road traffic accident

Of the 359 patients, the most frequent infection type is urinary tract infection, (n=146, 40.7%), followed by sepsis (n=72; 20.1%) and chronic wound infections (n=55; 13.3%). More than 95% of the patients used intravenous catheters either alone (78.6%) or with other invasive devices (17.0%). The major co-morbidities in the patients were hypertension (n=46; 12.8%), malaria (n=38; 10.6%), inguino-scrotal hernia (n=34; 9.5%) and diabetes mellitus (n=17; 4.7%). However, there was no co-morbidity in 56% of the patients. Eighty-five patients (23.7%) had at least one immunocompromised condition and ESBL bacteria were previously isolated in 3.3% of the patients. Other clinical details of the patients are shown in Table 2.

Of the total 359 non-duplicate GNB isolates recovered from different clinical specimens screened for ESBL production, 94 (26.2%) were ESBL producers, with *Escherichia coli* (n=25; 26.6%) as the commonest ESBL producer followed by *Citrobacter freundii* (n=23; 24.5%) and *Klebsiella pneumoniae* (n=13; 13.8%) (Table 3) with the most frequent ESBL producing GNB being *Stenotrophomonas maltophilia* 100% (2/2), followed by *Shigella dysenteriae* 66.7% (2/3), *Yersinia enterocolitica* 50% (2/4), *Enterobacter* sp 35.3% (12/34), *Klebsiella* sp 34.1% (14/41), *Citrobacter* sp 32.5% (27/83) and *Escherichia coli* 27.8% (25/84) while the least frequent ESBL producers are *Proteus* sp 11.1% (5/45), *Acinetobacter baumannii* 10% (1/10), *Pseudomonas aeruginosa* 2.6% (1/39) and *Hafnia alvei* 0%. The prevalence of ESBL-production was significantly lower among *Proteus* sp (OR = 0.3160, 95% CI = 0.1208-0.8268, p=0.0172) and *Pseudomonas* sp (OR=0.06423, 95%CI=0.0087-0.4749, $p<$ 0.0001) compared to other GNB isolates (Table 3).

Table 3: Prevalence of ESBL-producing Gram-negative bacilli infection and univariate analysis

GNB isolates	No of isolates	No of isolates producing ESBL (%)	Crude OR	95% CI	p value
Escherichia coli	84	25 (29.8)	1.265	0.7361 – 2.174	0.3978
Citrobacter sp	83	27 (32.5)	1.1504	0.8804 - 2.569	0.1545
Proteus sp	45	5 (11.1)	0.3160	0.1208 - 0.8268	0.0172*
Klebsiella sp	41	14 (34.1)	1.543	0.7710 - 3.087	0.2569
Pseudomonas aeruginosa	39	1 (2.6)	0.06423	0.0087 - 0.4749	<0.0001*
Enterobacter sp	34	12 (35.3)	1.616	0.7660 - 3.411	0.2205
Morganella morganii	11	3 (27.3)	1.059	0.2749 - 4.080	1.000
Acinetobacter baumannii	10	1 (10.0)	0.3059	0.0382 - 2.448	0.4646
Yersinia enterocolitica	4	2 (50.0)	2.859	0.3968 - 20.597	0.2813
Shigella dysenteriae	3	2 (66.7)	5.739	0.5140 - 64.075	0.1690
Stenotrophomonas maltophilia	2	2 (100)	14.351	0.6822 - 301.92	0.0680
Hafnia alvei	3	0	NA	NA	NA
Total	**359**	**94 (26.2)**			

OR=Odds Ratio; CI=Confidence Interval; NA = Not Applicable; ESBL=Extended Spectrum Beta Lactamase; *=statistically significant; NA=Not applicable

Table 4: Prevalence of ESBL-producing Gram-negative bacilli infection and univariate analysis in relation to specimen types

Clinical samples	No of GNB isolates	No of ESBL producing isolates (%)	Crude OR	95% CI	P value
Urine	159	35 (22.0)	0.6745	0.4162 - 1.093	0.1174
Wound	105	29 (27.6)	1.110	0.6647 - 1.852	0.6939
Blood	46	11 (23.9)	0.8709	0.4228 - 1.794	0.8577
Sputum	28	10 (35.7)	1.634	0.7253 - 3.679	0.2633
CSF	10	3 (30.0)	1.215	0.3076 - 4.800	0.7256
Stool	7	4 (57.1)	3.881	0.8520 - 17.682	0.08
Joint aspirate	1	0	NA	NA	NA
Endocervical swab	1	1 (100.0)	8.519	0.3438 - 211.10	0.2618
Ear swab	1	0	NA	NA	NA
Sequestrum	1	1 (100.0)	8.519	0.3438 - 211.10	0.2618
Total	**359**	**94 (26.2)**			

GNB=Gram-negative bacilli; ESBL=Extended Spectrum Beta-Lactamase; OR=Odds Ratio; NA=Not Applicable

Most of the ESBL producing isolates were obtained from urine (n=35; 37.2%), wound (n=29; 27.6%) and blood (n=11; 11.7%), however in descending order, the frequency of ESBL isolation from the clinical samples is as follows; endocervical swab 100% (1/1), sequestrum 100% (1/1), stool 57.1% (4/7), sputum 35.7% (10/28), CSF 30% (3/10), wound 27.6% (29/105), blood 23.9% (11/46), urine 22% (35/159), joint aspirate 0% and ear swab 0% (Table 4). Comparing the frequency of ESBL producing GNB isolates with respect to age group of patients, gender, ward from where organisms were isolated, diagnosis of infection/disease and other clinical parameters, showed that there is no significant differences in ESBL production with respect to type of specimen, diagnosis, site of infection, admission in previous year, previous use and type of antibiotics, duration of antibiotic treatment, and length of admission (*p*>0.05) (Tables 4 & 5).

However, from univariate analysis shown in Table 5, the prevalence of clinical infection by ESBL-producing bacteria was significantly higher (OR=1.90, 95% CI=1.18-3.06, *p*=0.0114) in male (32.7%, 55/168) compared with the female patients (20.4%, 39/191). The prevalence was also significantly higher (OR=2.13, 95% CI=1.23-3.69,

p=0.0103) in patients' age above 60 years (38.9%, 28/72) than other age groups. In the same vein, significantly higher prevalence rates were seen in patients admitted into intensive care unit (*p*<0.001), male surgical ward (*p*<0.001), farmers (*p*=0.0191) and retirees (*p*=0.0385). Significantly higher prevalence of ESBL were also reported in patients who used intravenous line (IVL) alone, or IVL with urocatheter, or IVL with endotracheal tube and urocatheter (*p*<0.001). In addition, presence of co-morbidities such as head injury, hypertension, benign prostatic hyperplasia (BPH), diabetes mellitus (DM) and immunocompromised states were all significantly associated with prevalence of ESBL-producing organisms (*p*<0.05) in the univariate analysis.

The results of multivariate logistic regression analysis are shown in Table 6. Apart from hypertension (OR=1.57, 95% CI=0.685-3.637, *p*=0.2846) and head injury (OR = 2.226, 95% CI = 0.9006 - 5.499, *p*= 0.084) which were not significantly associated with the prevalence of ESBL in the logistic regression analysis, all other variables such as male gender, admission to intensive care unit or male surgical ward, patients' occupation (farming, retired), presence of multiple invasive devices (IVL, endotracheal tube

and urocatheter, or IVL and urocatheter), underlying co-morbidities such as diabetes mellitus and benign prostatic hyperplasia (BPH), and immunocompromised state, were independent risk factors associated with the prevalence of ESBL GNB in this study.

Table 5: Predictors of ESBL producing Gram-negative bacilli infection by univariate analysis

Variables	ESBL (%) n=94 (26.2)	Non-ESBL (%) n=265 (73.8)	Crude OR	95% CI	p value†
Male gender	55 (32.7)	113 (67.3)	1.897	1.18 - 3.06	0.0114*
Age >60 years	28 (38.9)	44 (61.1)	2.131	1.232-3.686	0.0103*
Farming occupation	10 (50.0)	10 (50.0)	3.036	1.221-7.549	0.0191*
Retiree	12 (44.4)	15 (55.6)	2.439	1.10-5.42	0.0385*
Clinical diagnosis					
Chronic osteomyelitis	3 (60.0)	2 (40.0)	4.34	0.49-52.41	0.115
Gastroenteritis	3 (42.9)	4 (57.1)	2.15	0.31-12.95	0.384
Meningitis	3 (30.0)	7 (70.0)	1.22	0.20-5.46	0.726
Pneumonia	10 (35.7)	18 (64.3)	1.63	0.65-3.90	0.263
Sepsis	20 (27.8)	52 (72.2)	1.11	0.59-2.03	0.765
Surgical site infection	10 (31.3)	22 (68.7)	1.31	0.53-3.03	0.529
UTI	31 (21.2)	115 (78.8)	1.07	0.64-1.77	0.804
Chronic wound infection	13 (23.6)	42 (76.4)	0.85	0.40-1.72	0.740
Site of infection					
Abdomen	11 (33.3)	22 (66.7)	1.46	0.78-2.20	0.405
Blood stream	20 (27.8)	52 (72.2)	1.07	0.58-1.92	0.886
Extremities	12 (22.6)	41 (77.4)	0.80	0.49-1.38	0.613
Respiratory tract	9 (36.0)	16 (64.0)	1.65	0.62-4.13	0.245
Urinary tract	32 (21.5)	117 (78.5)	1.09	0.65-1.81	0.713
Previous admission in the last 1 year	25 (23.8)	80 (76.2)	0.84	0.47-1.46	0.598
Previous ESBL culture in last 1 year	3 (25.0)	9 (75.0)	0.94	0.16-3.87	1.000
Previous antibiotic use in last 3 mths	20 (21.5)	73 (78.5)	0.71	0.42-1.17	0.274
Admission for longer than 1 month	7 (28.0)	18 (72.0)	1.10	0.38-2.89	0.816
Antibiotic treatment for > 1 week	22 (22.7)	75 (77.3)	0.77	0.43-1.37	0.418
Admission into ICU	11 (73.3)	4 (26.7)	8.65	1.23-3.69	0.0001*
Admission into MSW	24 (54.5)	20 (45.5)	4.2	2.19 - 8.05	0.0001*
IVL	56 (19.9)	226 (80.1)	0.254	0.15-0.43	0.0001*
IVL, endotracheal tube, urocatheter	8 (88.9)	1 (11.1)	24.558	3.03-199.26	0.0001*
IVL, urocatheter	25 (58.1)	18 (41.9)	4.972	2.564-9.641	0.0001*
Head Injury	4 (80.0)	1 (20.0)	11.73	1.294-106.41	0.0179*
Hypertension	21 (45.7)	25 (54.3)	2.762	1.46 – 5.22	0.0021*
BPH	6 (54.5)	5 45.5)	3.545	1.056-11.907	0.0401*
Diabetes Mellitus	6 (60.0)	4 (40.0)	4.449	1.227-16.135	0.0231*
Immunocompromised states	30 (35.3)	55 (64.7)	1.790	1.058-3.028	0.0342*

†Fisher exact test for dichotomous predictors, MSW= male surgical ward, ICU =intensive care unit, IVL = intravenous line, BPH = benign prostatic hyperplasia; OR = Odds Ratio; CI = Confidence Interval; ESBL= Extended Spectrum Beta Lactamase, UTI = urinary tract infection, *statistically significant

Table 6: Independent predictors of ESBL-producing Gram-negative bacilli infection in multivariate logistic regression model

Predictor	Adjusted OR	95% CI	p value
Male gender	1.131102	1.033234 - 1.238240	0.00798*
Age >60years	3.264896	2.315363 - 4.603835	0.0225*
ICU	1.7623826	1.2886753 - 2.410221	0.000443*
MSW	1.4605132	1.1224911 - 1.900326	0.005078*
Farming	1.3448686	1.0755175 - 1.681676	0.009800*
Retiree	1.2721912	1.0402855 - 1.555794	0.019660*
IVL, Endotracheal Tube, Urocatheter	1.9943284	1.5203033 - 2.616153	$9.74e^{-7}$*
IVL/Urocatheter	1.4664049	1.2860782 - 1.672016	$2.31e^{-8}$*
Head Injury	2.225541	0.9006394 - 5.499462	0.0840
Hypertension	1.578574	0.6850692 - 3.637435	0.2846
BPH	1.725392	1.2017102 - 2.477285	0.00335*
Diabetes Mellitus	1.822119	1.2583221 - 2.638527	0.00164*
Immunocompromised states	1.126781	1.012884 - 1.253484	0.0288*

ICU- intensive care unit, MSW- male surgical ward, IVL- intravenous line, BPH- benign prostatic hyperplasia; OR = Odds Ratio; CI = Confidence Interval; *=statistically significant

Discussion:

In this study, we identified some risk factors for ESBL-producing bacterial infection by univariate and multivariate logistic regression analyses. Age greater than 60 years, male gender, admission to the intensive care unit (ICU) and male surgical ward, patients' occupation (farmers and retirees), use of IVL, endotracheal tube, and urocatheter, underlying illnesses such as DM, BPH, immunocompromised state of the patients were identified as independent risk factors for ESBL infection. Only a few studies have investigated the risk factors for ESBL-GNB infections in a tertiary care hospital in this environment. Therefore, this study adds new information to the knowledge gap that exists in this area of clinical care.

High prevalence of ESBL-producing bacteria among retirees and patients aged 60 years and above noted in our study has previously been reported by Musikatavorna et al., (16) among bloodstream infection cases in Thailand. This can be explained by the increase in frequency and duration of hospital admissions usually associated with this advanced age category as well as the immune system fragility of these elderly patients, making them more prone to clinical infection, leading to increase antimicrobial use (17).

Uropathogens contributed the largest number of uropathogens while prevalence for ESBL-producing bacteria is highest in stool (57.1%), followed by sputum (35.7%) and wound (27.6%). This study also showed that BPH and admission into surgical wards are predictors of infection by ESBL-producing bacteria. Complicated UTI in men is associated with structural or functional abnormality in the urinary tract, often requiring prolonged antibiotic treatment (18). All these factors make male patients with BPH admitted to surgical wards more prone to acquiring multidrug resistant bacteria (19). These findings had been well noted in an Asian study which reported high prevalence of ESBL-producing

pathogens as agents of UTI especially among surgical patients (9). Similar to our findings, a study in Mexico also reported urological abnormalities (OR=3.88, 95% CI 1.31-11.47, p=0.005), and urinary catheterization (OR = 3.90, 95% CI = 1.13 - 14.08, p = 0.008) as factors significantly associated with acquisition of ESBL-producing uropathogens (20). Likewise, high prevalence of ESBL-producing bacterial infection in surgical wards was also established in a hospital-based case control study among Swedish population (9). Though a higher prevalence of ESBL-producing bacteria was found in male patients compared to female patients in our study, other researchers have reported significantly higher rate in the female gender (21), hence it is expedient that clinicians look out for factors predisposing factors to infection by ESBL-producing bacteria in individual patients irrespective of their age and gender.

It has been shown in this study and those of others (22,23) that patients admitted to ICU were more likely to have infections by ESBL-producing bacteria compared to patients in the other wards. It is a common knowledge that ICU is a hotspot for antibiotic-resistance and this is attributed to excessive use of broad-spectrum antimicrobial agents such as third-generation cephalosporins, vancomycin and imipenem, which have a higher propensity for selecting antimicrobial-resistant bacteria (24). Also, the specific risk profiles of patients coupled with multiple procedures and use of invasive devices (such as intubation, mechanical ventilation, vascular access) makes ICU the epicentre of resistance development. Among critically ill patients, invasive devices/procedures like central venous line, mechanical ventilation and stomach tube catheterization constituted significant risks for infections by the drug-resistant pathogens (6,25). Worse still for these patients, commencement of appropriate antibiotic therapy is often delayed with grave consequences. Delay in effective treatment of patients with systemic infe-

Risk factors for ESBL-producing Gram negative bacterial infections

Afr. J Clin. Exper. Microbiol. 2022; 23 (2): 149-158

ction caused by ESBL-producing bacteria has far reaching prognostic implications, patients often become clinically critical necessitating ICU care in many cases which further reduces their chances of survival. A study done in the UK revealed a significant delay in instituting appropriate therapy for cases of ESBL-producing bacterial infections (OR 9.17, 95% CI 2.00 - 42.20, *p*=0.0005) with survival estimates demonstrating a significantly increased early (<25 days after infection) mortality (OR for death 3.93, 95% CI 1.05-14.63, *p*=0.03) (35). Admission into ICU is a documented risk for death from bacteraemia caused by ESBL-producing pathogens (26).

Our study further revealed immuno-compromised states including DM as risks for ESBL-producing bacterial infections. Silva et al., (7) had reported similar findings in Brazil in which malignancy and DM independently predisposed patients to nosocomial infections caused by ESBL-producing *Klebsiella pneumonia*. Similarly, in a study conducted in a paediatric tertiary hospital in Bangkok Thailand, prevalence of ESBL infection was reported to be higher among patients with immuno compromised conditions, especially haematologic malignancies than among patients without underlying disease. Although the development of antimicrobial resistance is a natural phenomenon, immuno-compromised conditions such as DM predispose patients to repeated and multiple infections making excessive use of antimicrobials inevitable, and thus contributing to emergence and spread MDR organisms including ESBL-producing bacteria (27). Immunocompromised conditions such as AIDS make it possible for patients to become reservoirs of MDR opportunistic pathogenic organisms with vast abilities for horizontal dissemination (28).

Our study also identified an association between farming occupation and infection by ESBL-producing pathogens. Farmers in this environment are generally peasant who are mostly down in the socioeconomic ladder, and lack of money to facilitate appropriate antimicrobial treatment is one of the major poverty-driven factors contributing to AMR among them (29). These patients may only complete a truncated course of therapy because of their inability to pay for the full course of medications. Widespread neglects of health financing is responsible for persistence of user fees as mainstay of health financing manifesting in increasing out-of-pocket expenditures which further aggravates poverty in-country (30).

The limitations of our study include being a cross-sectional design, it did not allow determination of cause-effect relationship, and also data were collected from case folders of participating patients with the pos-

sibility of incomplete record.

Conclusion:

In conclusion, our study revealed that elderly, male gender and farming as well as admission into male surgical ward and intensive care unit are predisposing factors for acquisition of ESBL-producing bacteria. Other identified risks are use of invasive devices, benign prostatic hyperplasia and immuno-compromised states including DM. Identifying these factors provides the basis for infection prevention and control interventions as well as protocols for improved antibiotic use to strengthen antimicrobial stewardship in our hospital setting.

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Audit Report   **Open Access**

Baseline health facility assessment of quality assurance for malaria diagnosis in existing government hospital laboratories in Sokoto State, Nigeria

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Abstract:

Background: Quality laboratory services are needed to direct reasonable malaria case management through malaria microscopy and rapid diagnostic test. This study assessed the existing diagnostic capacities including laboratory structures and systems, human resource, skills and competences, using the standardized WHO/NMEP EQA assessment tool.
Methodology: Data were collected by an assessment team using a standardized assessment instrument/checklist drawn from WHO/NMEP assessment tool and analyzed with Open Data Kit (ODK) and Open-source suite of tools on Android mobile devices from September 3-11, 2020. The use of ODK allowed data to be collated offline where internet services were poor or unavailable and uploaded thereafter.
Results: Of the 24 laboratory facilities assessed, diagnostic services on malaria are routinely done with combined malaria rapid diagnostic test (mRDT) and microscopy at 17 (65.0%) laboratories, microscopy only at 7 (27.0%) laboratories, while only mRDT was performed in 1 (3.8%) laboratory due to lack of functional microscopes, supplies, or trained personnel in microscopy. In the 24 facilities providing laboratory services, 16 (63.0 %) had one of the staff received basic malaria microscopy and mRDT training in the year prior to the assessment, and 23 (96.0%) of the laboratories had at least one functional electric binocular microscope. None of the laboratory had a good structured quality assurance/quality control procedure or standard operating procedures for either microscopy or mRDT.
Conclusion: There were gaps in laboratory services due to lack of well-established quality control framework and ineffective communication system, which could have substantial impacts on the quality and accessibility of malaria diagnosis. These issues can be addressed by improving laboratory services.

Keywords*: malaria; quality assurance; hospital laboratory; secondary health facilities; capacity

Évaluation de base par les établissements de santé de l'assurance qualité pour le diagnostic du paludisme dans les laboratoires hospitaliers gouvernementaux existants dans l'État de Sokoto, au Nigéria

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Résumé

Contexte: Des services de laboratoire de qualité sont nécessaires pour diriger une prise en charge raisonnable des cas de paludisme grâce à la microscopie du paludisme et au test de diagnostic rapide. Cette étude a évalué les capacités de diagnostic existantes, y compris les structures et les systèmes de laboratoire, les ressources humaines, les aptitudes et les compétences, à l'aide de l'outil d'évaluation EQA standardisé de l'OMS/NMEP.

Méthodologie: Les données ont été collectées par une équipe d'évaluation à l'aide d'un instrument d'évaluation/liste de contrôle standardisée tirée de l'outil d'évaluation de l'OMS/NMEP et analysées avec Open Data Kit (ODK) et une suite d'outils open source sur les appareils mobiles Android du 3 au 11 septembre 2020 L'utilisation d'ODK a permis de rassembler les données hors ligne lorsque les services Internet étaient médiocres ou indisponibles et de les télécharger par la suite.

Résultats: Sur les 24 établissements de laboratoire évalués, les services de diagnostic du paludisme sont systématiquement effectués avec un test de diagnostic rapide du paludisme combiné (mRDT) et une microscopie dans 17 laboratoires (65,0%), la microscopie uniquement dans 7 laboratoires (27,0%), tandis que seul le mRDT a été effectué. dans 1 (3,8%) laboratoire en raison du manque de microscopes fonctionnels, de fournitures ou de personnel qualifié en microscopie. Dans les 24 établissements fournissant des services de laboratoire, 16 (63,0 %) avaient un membre du personnel ayant reçu une formation de base en microscopie du paludisme et mRDT au cours de l'année précédant l'évaluation, et 23 (96,0%) des laboratoires avaient au moins un microscope binoculaire électrique fonctionnel. Aucun des laboratoires ne disposait d'une bonne procédure structurée d'assurance qualité/contrôle qualité ou de modes opératoires normalisés pour la microscopie ou le mRDT.

Conclusion: Il y avait des lacunes dans les services de laboratoire en raison de l'absence d'un cadre de contrôle de la qualité bien établi et d'un système de communication inefficace, ce qui pourrait avoir des impacts substantiels sur la qualité et l'accessibilité du diagnostic du paludisme. Ces problèmes peuvent être résolus en améliorant les services de laboratoire.

Mots clés: paludisme; assurance qualité; laboratoire hospitalier; établissements de santé secondaires; capacité

Introduction:

The 2020 World Malaria Report estimates that 229 million cases of malaria occurred worldwide with approximately 409,000 deaths (1). The most vulnerable malaria-affected group were children under the age of 5 years who accounted for 67% (272,000) of all deaths from malaria worldwide. Nigeria alone accounts for 27% of the world malaria cases (1). Over 97% of the projected 5.4 million people in Sokoto State are at risk of malaria infection every year caused by *Plasmodium falciparum*. The main determinants of malaria epidemiology in the State are rainfall, environmental factors, and human status, and with a childhood mortality of over 19 per 100,000 population, the disease still ranks as the highest cause of deaths amongst children and places a huge burden on the economy in terms of man-hours lost in business and schools (2).

In Sokoto State, Nigeria, most secondary health facilities are primarily concentrated in urban and semi-urban areas, whereas primary health care (PHC) centers are situated in rural areas and are usually staffed by at least one community health officer, nurse, laboratory technicians, pharmacists or midwife. Rural area are settlements with fewer than 2,500 residents while urban areas comprise larger places and densely populated areas around them, with semi-urban in between rural and urban, but not wholly characteristic of rural areas. Most patients with complicated malaria are evaluated and treated at secondary or tertiary health facilities.

The secondary health facilities have laboratory services that examine blood films under the microscope, whereas the PHCs rely on malaria rapid diagnostic tests (mRDTs).

High-quality laboratory services for the diagnosis of malaria are not widely available in Sokoto State, especially at the community level, where access to accurate malaria diagnosis and appropriate treatment are inadequate. Both the WHO policy on malaria diagnostics and the National Guidelines for Diagnosis and Treatment of malaria recommends the use of microscopy and quality-assured mRDTs for parasitological diagnosis of malaria. However, microscopy should be deployed at tertiary and secondary health facilities while mRDTs at the PHC facilities, and supported by quality assurance (QA) program, since microscopy remains the "gold standard" for parasitological diagnosis of malaria. Although molecular techniques such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) assays have higher sensitivity and specificity, they are mostly research tools, and are not used for routine diagnosis due to high cost and technical complexity.

The mainstay of an efficient, effective, and accurate diagnosis of malaria revolves around putting in place QA program. In Sokoto State, there had been few research publications on malaria (3,4) but there is probably no published QA information concerning the study area across the tiers of health care system. The introduction of QA system is therefore an opportunity to review and strengthen the entire available

malaria diagnostic system for provision of a quality assured malaria parasite-based diagnosis in the State which are crucial in reducing misdiagnosis, over-diagnosis, quality-assured care, and reduce drug resistance and wastages due to wrong prescription of antimalarial.

The national guidelines for diagnosis and treatment of malaria recommends that all persons suspected for fever cases be tested with mRDT at PHC levels or microscopy at secondary and tertiary health facilities before treatment. The parasitological diagnosis should be quality assured (5). It is important to confirm the existence of the malaria parasite, as clinical diagnosis is often inaccurate, resulting in antimalarial drug waste and possibly contributing to drug resistance. Therefore, setting up an effective and functional QA system for both microscopy and mRDT is considered indispensable to ensuring accuracy and reliability of the diagnostic test result for effective malaria treatment. This is complemented by trained, competent and motivated staff, and supported by effective supervision to achieve reliable, accurate and timely malaria diagnosis.

To achieve this objective, the National Malaria Elimination Program (NMEP) has prioritized the development, design, and implementation of malaria diagnostic QA system in Nigeria. A comprehensive baseline study was conducted in Sokoto State to assess the existing malaria diagnostic capacities with the use of microscopy, including laboratory structures, human resource, skills and competences, waste disposal, equipment, commodity and reagents, availability of water, and storage facility, in order to ensure the accuracy and reliability of the diagnostic test result for effective malaria case management.

Materials and method:

Study setting, design and data collection tool

A profiling assessment on malaria diagnostic capacity was carried out in 24 out of 25 laboratories of public secondary health facilities across the 23 Local Government Areas (LGAs), covering the three Senatorial Districts of Sokoto State, Nigeria (Fig 1). This study used a purposive sampling technique to ensure total quality coverage of health facilities across the State, but the 25th heath facility could not be assessed due to security issues in the LGA at the time of the study. The study was adapted from the WHO Malaria Microscopy Quality Assurance Manual, Version 2, 2016 (6), and the Federal Ministry of Health National Malaria Elimination Program on Malaria Diagnostic External Quality Assurance Operational Guidelines 2018 tools (5).

Ethical approval

The study was approved by the Ethical Committee of Sokoto State Ministry of Health, and informed consent was obtained from the head of every medical facility assessed.

Data collection

A two-day training was conducted for the assessment team using the adapted tools from WHO and FMOH (5,6). The tool is health facility malaria microscopy QA checklist that; (i) describes health facility and laboratory, hours of operations, biosafety across the health facility; (ii) a logistic system required to ensure adequate, continuous supply of good-quality reagents, and essential equipment maintained in working order for microscopy services; (iii) human resources comprising competent and motivated staff, supported by effective training and supervision; (iv) laboratory infrastructures, standard operating procedures (SOPs) or bench aids; and (v) equipment and QA of malaria diagnosis.

Using an Android mobile device, Open Data Kit (ODK) platform deployed for data collection involves the following steps; form building, validation/testing, training, data collection, collation (aggregate and briefcase), and data analysis. The original assessment questionnaire was a 13-page assessment interviewer-administered tool in various subsections. However, this was expanded to incorporate introduction page, global positioning system (GPS) capturing of facility coordinates; facility information; information on facility characteristic, catchment population, kilometer radius, followed by response questions.

Data analysis

Field data were collected into an open-source Google data which was transcribed into Microsoft Excel 16 version dataset and analysed using Stata 12 (StataCorp, College Station, TX, USA).
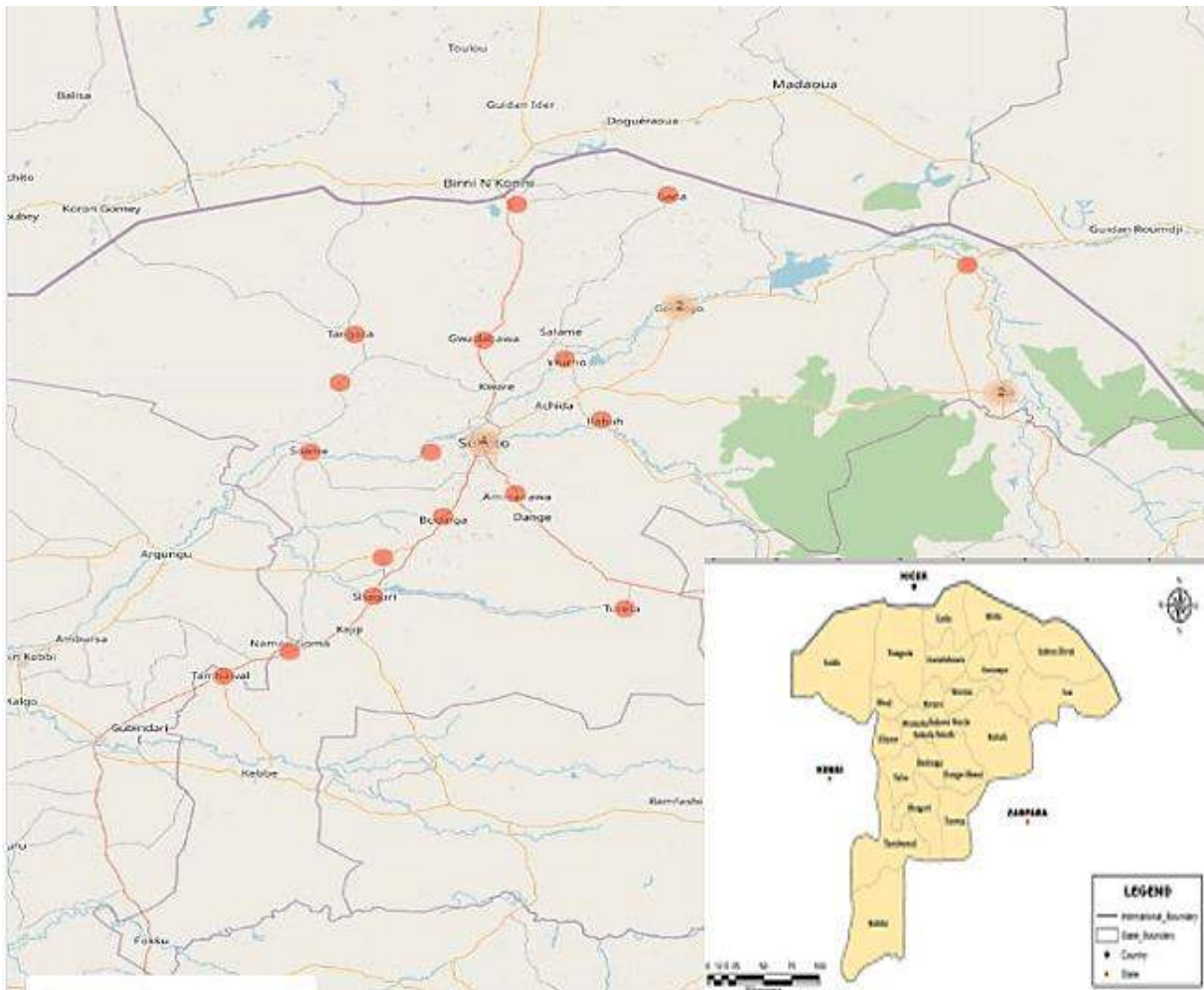
Fig 1: Location and distribution of health facilities surveyed in Sokoto State, Nigeria

Results:

The location of the facilities determines availability of basic amenities required for effective functioning of a laboratory. Majority of the health facilities (80%) were typically in semi-urban settings often without electricity while 20% are in urban centers. There was marked variability in the laboratory diagnostic capacities among the facilities assessed.

Regarding competency, 16 (63.0%) laboratories had one scientist who had received basic malaria microscopy training, but with none of the scientists passing the National certification (equivalent to WHO certification) in microscopy and mRDT training in the year prior to the assessment. Twenty-three (96.0%) of the facilities had at least one functional electric binocular microscope. The facilities had variable levels of equipment, materials, and biosafety procedures necessary for laboratory diagnosis of malaria.

None of the assessed laboratory facility had a good formal quality assurance/quality control protocols for either microscopy or mRDTs.

Characteristics of health facilities assessed

The median catchment population was 350,000 people (range of 154,709 - 1,255,709) with a geographical kilometer in radius mean standard deviation of 65±47 km (the standard deviation calculation of distances between points, which are defined by geographical coordinates in terms of latitude and longitude to Sokoto, the State capital).

The hours of operation were 24 hours per day in 22 (92.0%) of the assessed facilities and 8am-4pm per day for 7 days a week in 2 (8.3%) facilities. Electricity was available in all (100%) the facilities through; generator (89%), solar (42%), and inverter (26%), while (38%) was from the national electricity grid.

Of the 24 assessed health facility laboratories, 10 (42.0%) do have electricity interruptions interfering with the ability to perform malaria microscopy. Only 6 (23.0%) health facilities had ready access to running piped water, with borehole/well water in 22 (85.0%). Most laboratories have leaking roofs (Fig. 2), and only 6 (25.0%) facility laboratories had floor tiles while there were no floor tiles in 19 (75.0%) laboratory facilities.

Human resources and training

From the assessed laboratory facilities, poor personnel distribution and general management was observed. As a result, some facilities are over staffed while other are under-staffed with no malaria EQA National certified malaria microscopist (Fig. 3). There were many unqualified personnel (17.0% of the total laboratory personnel, n=80) in the assessed health facilities performing malaria microscopy.

Fig 2: Laboratory without benches and with dilapidated equipment and leaking roof in selected health facilities, Sokoto State, Nigeria
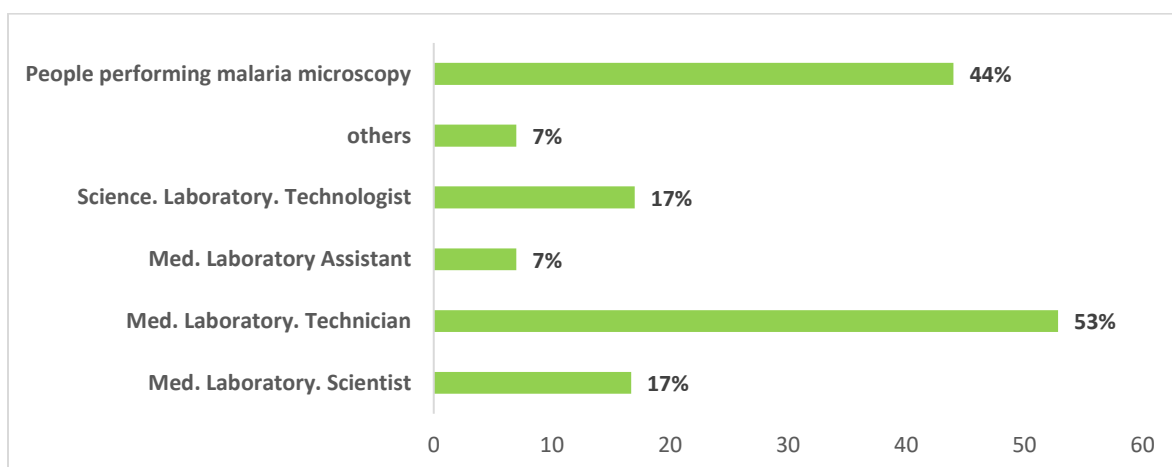
Fig 3: Distribution of Medical Laboratory Scientists, Medical Laboratory Technicians, Medical Laboratory Assistants and other technical staff in assessed heath facilities, Sokoto State, Nigeria

163

Biosafety and laboratory operations

Majority (92.0%, n=24) of the laboratory staff use hand gloves and protective coats when in the laboratory facilities. Hand-washing facilities were available for staff where malaria diagnosis was performed in 21 (81.0%) facilities. The use of different disinfectants and anti septics was observed. Over 70% (n=17) of the laboratories do not segregate waste appropriately, with only 7 (27.0%) using colored-coded buckets waste bins for storing both contaminated sharp and contaminated non-sharp wastes (e. g. used plastic wares, gloves and swabs) (Fig 4a).

Immediate disposal of contaminated sharp wastes was encouraging as obtained in 85% of the health facilities. However, the final method of waste disposal for contaminated sharps, non-sharps, blood, and blood products was mainly open field burning (88.0%) with only 23.0% and 12.0% using incinerator and pit burial respectively (Fig 4b).

Fig 4a: Methods used for sharp collection in assessed heath facilities, Sokoto State, Nigeria

Fig 4b: Disposal systems at assessed health facilities showing pit disposal system
and waste storage at one of the assessed laboratories

164

Fig 5: Malaria diagnostic services routinely available at health facilities, Sokot State, Nigeria

Malaria diagnosis

Of the 24 health facility laboratories reportedly performing malaria diagnostic services routinely, combined mRDTs and microscopy was performed in 16 (67.0%), microsc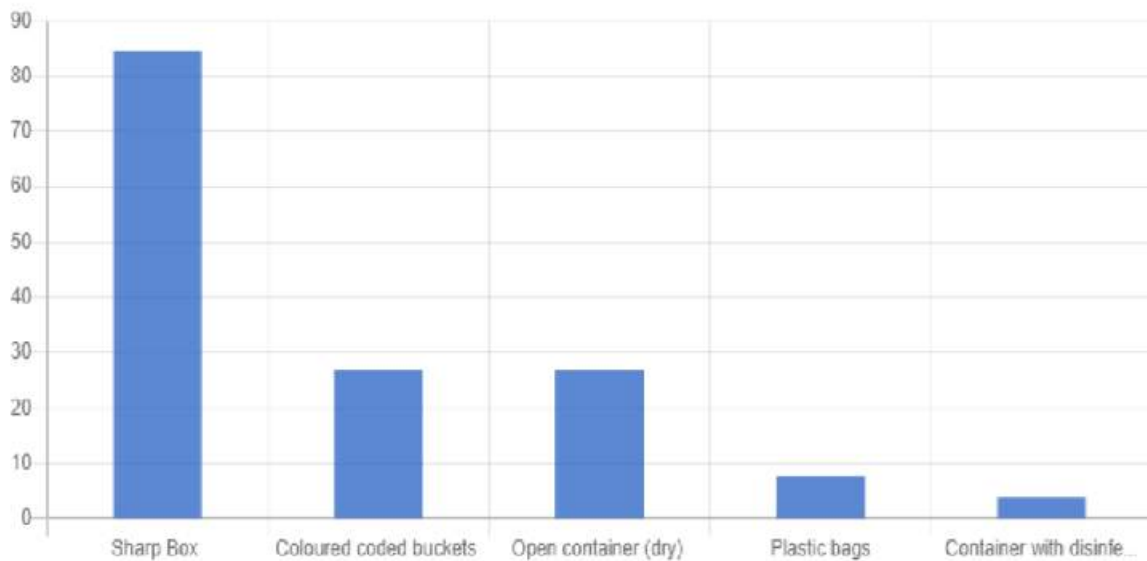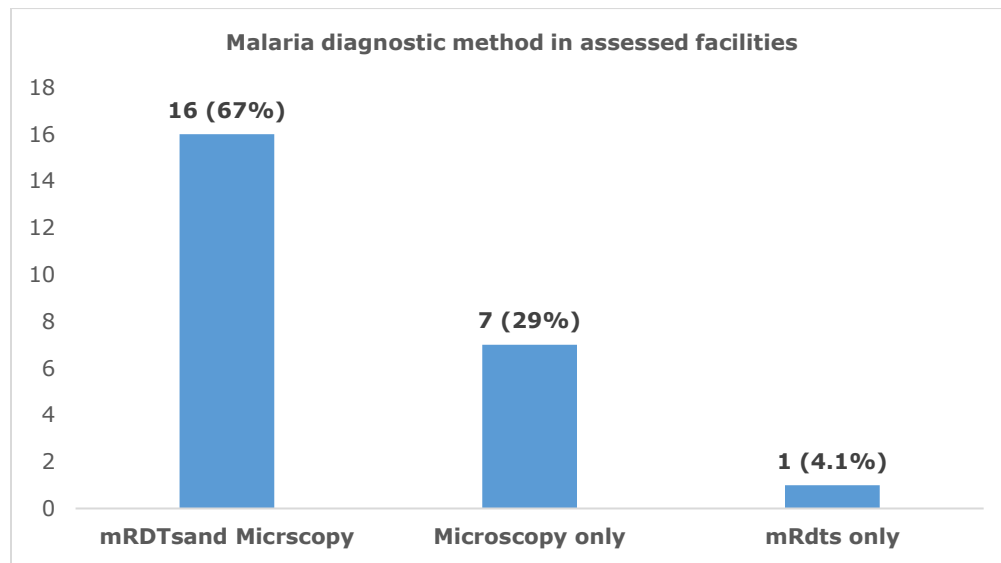opy only in 7 (29%), and mRDTs only in 1 (4.1%). Reasons for non-availability of microscopic diagnosis includes lack of functional microscopes, supplies, or lack of personnel trained in microscopy (Fig. 5). Malaria RDTs were reportedly used in place of microscopy during busy laboratory working conditions when additional microscopy workload would cause excessive delays in clinical management of patients, and when laboratory staff were not available.

Among the facility laboratories assessed, 15 (62.0%) employed only thick blood films routinely for malaria diagnosis, while 9 (38.0%) employed both thick and thin blood films, with 1 (3.8%) facility laboratory employing only thin film for malaria diagnosis. Also, 20 (83.0%) facility laboratories used Giemsa stain for malaria blood films, while 2 (8.0%) used both Giemsa and field stain and 2 (8.0%) used only field stain.

Major malaria laboratory equipment

Availability and functionality of equipment was equally assessed. Twenty-three (96%) of the assessed facility laboratories had at least one WHO recommended electric binocular microscope and 1 (4.1%) had one daylight binocular microscope using the phone touch light as the light source (Fig. 6a) because there was no spare bulb to replace the burnt microscope bulb. Among the facility laboratories with binocular microscopes, only 2 (8.3%) reported having spare microscope bulbs, but we observed slide staining and drying racks in all (100%) facilities.

There were no benches in 3 (12%) laboratories, as a result, microscopes were kept on chairs or ordinary tables (Fig. 6b), and malaria staining done in the toilet (Fig. 6c), and outside the laboratory (Fig.6d). There was no water distillation system in 16 (67.0%) assessed facility laboratories, while 8 (33.0%) have one in place.

Fig 6 showing; (a) a technician examining a slide using touch light of a mobile phone as source of light to the microscope; (b) microscope kept on a chair, (c) toilet used as staining basin due to lack of a staining rack, and (d) staining for malaria parasites done outside the laboratory

Fig 7: A store without cabinets, leaking ceiling of a store, and consumables/supplies on the floor of a store in one of the selected health facilities, Sokoto State, Nigeria

Consumables, storage, and equipment maintenance

Of the assessed facility laboratories, 23 (95.0%) reported getting their supplies of Giemsa stain from local suppliers while 1 (4.2%) reported getting its supplies from Sokoto State Drug Revolving Fund. Reportedly, the assessment indicated shortage problems with supply of lancets, immersion oil, lens tissue, lead or grease pencils, alcohol, and bleach in all the facilities. It was also observed that staff were not trained on equipment maintenance in all the assessed facilities. Also, there was no good storage facility for supplies and commodities in all the laboratories and where there were storage facilities, there were no cabinets for storing reagents or mRDTs (Fig. 7).

Quality Assurance (QA) practices

In terms of QA practices, findings from the assessment show that 19 (79.0%) facility laboratories did not perform equipment calibration and maintenance as per standard instruction. Almost all (88.0%) of the laboratories also indicated that there were supplies and reagents interruption and where supplies and reagents are available, there were poor quality staining outputs. Likewise, internal quality control (IQC) was not conducted regularly.

QA protocol in place for malaria microscopy was not available in 22 (92.0%) assessed laboratories while 2 (8.3%) had QA protocol in place. There was no microscopy or mRDT training or QA training provided to the assessed facility laboratories in the past 12 months. In 4 (17.0%) laboratories, no SOP was available, while 22 (83.0%) had SOPs in place. In addition, 5 (21.0%) did not have bench aids for malaria procedures, while 19 (79.0%) had bench aids in place.

Discussion:

Many countries across the sub-Saharan Africa are scaling up malaria prevention and control measures such as mass distribution of long-lasting insecticide-treated nets, indoor residual spraying of households, and provision of early diagnosis and treatment in response to the Global Malaria Action Plan (GMAP) (Roll Back Malaria Alliance RBMA, 2008) and increased global commitment to malaria prevention and control programs. Presumptive malaria care should be avoided, according to the WHO and GMAP, all suspected malaria cases in all age groups should be verified by laboratory diagnosis (6).

Knowing that laboratory services play important role in the diagnosis of infectious diseases such as malaria, most of the laboratories in most heath facilities assessed had dilapidated laboratory infrastructures. We discovered high workload in some facilities, shortage of resources, poor management supports, poor staff motivation, lack of training, high number of faulty equipment and failure, shortage of supplies and reagents were the main factors impacting the laboratory QA practices. Some of the laboratory professionals do not practice IQC activities and do use SOPs. Poor personnel distribution and general management issues were observed, and as a result, some facilities are over staffed while others are understaffed.

There was also poor quality malaria diagnosis coupled with lack of mentorship, training and retraining for the scientists and technicians/assistants; poor quality and insufficient number of light microscopes at all levels of healthcare, high prevalence of sub-standard reagents for malaria microscopy, logistic problems and high costs of maintaining adequate supplies and equipment; inadequate provision of malaria diagnostic equipment and commodities; insufficient number of trained malaria microscopists and lack of effective QA system. There were variety of issues, including inconsistent and erratic electricity supply, security issues, and lack of storage systems for laboratory supplies. Clearly, some of the needs and gaps identified such as supply of gloves and laboratory coats should be

easily addressed than others such as access to water and electricity, adequately trained and motivated staff, and proper waste disposal management, which may require having more substantial policy and financial support.

While our baseline assessment indicated that laboratory diagnosis of malaria, either by microscopy or mRDTs, was available in all (100%) the facilities assessed, the assessment also revealed major deficiencies in laboratory service functions such as laboratory infrastructures, equipment, materials, and human resources, which could have effect on the quality and accessibility of malaria diagnosis. The data provided in the study could be useful in preparation, budgeting, and developing strategies to increase malaria laboratory capability. Even at the community level, dependable techniques exist to enable accurate malaria diagnosis (7), and distinct stages of the healthcare delivery system may have different diagnostic needs (8). It is also clear that improving malaria diagnosis is critical to improving a country's laboratory services as a whole (9,10).

Effective and reliable laboratory malaria diagnosis is a major challenge across all our secondary facilities in Sokoto State and our assessment revealed that laboratory diagnosis of malaria, either by microscopy or mRDTs, was available in most of the facilities assessed. However, it also revealed significant gaps in the laboratory service functions, such as laboratory infrastructure, equipment, poor human resource management, poor resources provision, poor management commitment, lack of supplies and faulty equipment, ineffective communication system and lack of well-established quality management system (QMS).

To address these gaps, there is urgent need for proper planning, provision of required materials, equipment, and recruitment of qualified manpower and capacity building of the existing staff as findings from this assessment should be used to design and implement activities to strengthen laboratory capacity for malaria diagnosis in Sokoto State. To ensure that all laboratories have reagents and materials, facilities with non-functional microscopes have to be fitted with microscopes to ensure that blood slide examination is not interrupted, Medical laboratory workers from these facilities need to be re-trained in malaria diagnosis including basic malaria microscopy, RDTs, laboratory protection, and QA and QC by strengthening laboratory services through urgent planning, provision of required materials, equipment, and recruitment of qualified manpower and capacity building of the existing staff and need for

adequate funding and the implementation of a QA system.

Conflict of interest:

Authors declare no conflict of interest

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Original Article   **Open Access**

Prevalence of Macrolide-Lincosamide-Streptogramin-B resistance among clinical *Staphylococcus aureus* isolates in University of Ilorin Teaching Hospital, Ilorin, Nigeria

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Abstract:

Background: Inducible antibiotic resistance among Gram-positive cocci is a significant public health challenge that is grossly underreported within Africa, especially Nigeria. Hence, the aim of this study was to determine the prevalence of macrolide-lincosamide-streptogramin-B (MLSB) resistance among clinical isolates of *Staphylococcus aureus* at University of Ilorin Teaching Hospital, Ilorin, Nigeria.
Methodology: Clinical isolates were presumptively identified by Gram's stain reaction and conventional biochemical tests such as catalase, coagulase, DNase, and mannitol fermentation. Phenotypic MLSB resistance was determined by placing clindamycin and erythromycin discs within 15 mm of each other and observing for a D-zone. Antibiotic sensitivity testing to selected antibiotics including cefoxitin for detection of methicillin resistance, was done using the modified Kirby-Bauer disc diffusion method.
Results: Of the total 112 *S. aureus* isolates tested in the study, 31 (27.7%) were MLSB-resistant. MS phenotype (16.1%) was the most prevalent phenotype followed by constitutive MLSB (cMLSB) resistance (6.2%), and inducible MLSB (iMLSB) resistance (5.4%). All MLSB-resistant and sensitive *S. aureus* isolates were susceptible to linezolid, rifampin, tigecycline, and mupirocin while resistance rates of the MLSB resistant isolates (n=31) to other antibiotics were; tetracycline (58.1%), ciprofloxacin (48.4%), fusidic acid (41.9%), gentamicin (38.71%), cotrimoxazole (35.5%), fosfomycin (29.0%), and cefoxitin (70.9%). Comparatively, resistance rates of the MLSB-sensitive isolates (n=81) to other antibiotics are; tetracycline (70.4%), ciprofloxacin (39.5%), fusidic acid (22.2%), gentamicin (45.7%), cotrimoxazole (46.9%), fosfomycin (18.5%) and cefoxitin (34.6%). There was no significant difference in the antibiotic resistance rates between MLSB resistant and MLSB sensitive strains to the antibiotics ($p > 0.05$) except to fusidic acid ($p = 0.0369$) and cefoxitin ($p < 0.0001$). There was also no significant difference in antibiotic resistance rates with respect to the three MLSB resistance phenotypes ($p > 0.05$), except for fusidic acid which was significantly higher in cMLSB than other phenotypes ($p = 0.007$).
Conclusion: The introduction of MLSB resistance detection among Gram-positive cocci in routine microbiological practice can play an important role in monitoring inducible resistance and thereby preventing therapy failure.

Keywords: *Staphylococcus aureus;* D test; constitutive MLSB; inducible MLSB; MS phenotype; resistance

Prévalence de la résistance au macrolide-lincosamide-streptogramine-B parmi les isolats cliniques de *Staphylo-coccus aureus* à l'hôpital Universitaire de l'Université d'Ilorin, Ilorin, Nigeria

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Prevalence of MLS$_B$ resistance in Staphylococcus aureus

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Abstrait:

Contexte: La résistance inductible aux antibiotiques chez les cocci à Gram positif est un défi de santé publique important qui est largement sous-déclaré en Afrique, en particulier au Nigeria. Par conséquent, le but de cette étude était de déterminer la prévalence de la résistance au macrolide-lincosamide-streptogramine-B (MLS$_B$) parmi les isolats cliniques de *Staphylococcus aureus* à l'hôpital universitaire d'Ilorin, Ilorin, Nigeria.

Méthodologie: Les isolats cliniques ont été identifiés par présomption par la réaction de coloration de Gram et des tests biochimiques conventionnels tels que la catalase, la coagulase, la DNase et la fermentation du mannitol. La résistance phénotypique au MLS$_B$ a été déterminée en plaçant des disques de clindamycine et d'érythromycine à moins de 15 mm l'un de l'autre et en observant une zone D. Les tests de sensibilité aux antibiotiques pour certains antibiotiques, y compris la céfoxitine, pour la détection de la résistance à la méthicilline, ont été effectués à l'aide de la méthode de diffusion sur disque de Kirby-Bauer modifiée.

Résultats: Sur les 112 isolats de *S. aureus* testés dans l'étude, 31 (27,7%) étaient résistants à la MLS$_B$. Le phénotype MS (16,1%) était le phénotype le plus répandu, suivi de la résistance constitutive au MLS$_B$ (cMLS$_B$) (6,2%) et de la résistance inductible au MLS$_B$ (iMLS$_B$) (5,4 %). Tous les isolats de S. aureus résistants et sensibles au MLS$_B$ étaient sensibles au linézolide, à la rifampicine, à la tigécycline et à la mupirocine, tandis que les taux de résistance des isolats résistants au MLS$_B$ (n=31) à d'autres antibiotiques l'étaient; tétracycline (58,1%), ciprofloxacine (48,4%), acide fusidique (41,9%), gentamicine (38,7%), cotrimoxazole (35,5%), fosfomycine (29,0%) et céfoxitine (70,9%). Comparativement, les taux de résistance des isolats sensibles au MLS$_B$ (n=81) à d'autres antibiotiques sont; tétracycline (70,4%), ciprofloxacine (39,5%), acide fusidique (22,2%), gentamicine (45,7%), cotrimoxazole (46,9%), fosfomycine (18,5%) et céfoxitine (34,6%). Il n'y avait pas de différence significative dans les taux de résistance aux antibiotiques entre les souches résistantes au MLS$_B$ et les souches sensibles au MLS$_B$ aux antibiotiques ($p>0,05$) sauf à l'acide fusidique ($p=0,0369$) et à la céfoxitine ($p<0,0001$). Il n'y avait pas non plus de différence significative dans les taux de résistance aux antibiotiques par rapport aux trois phénotypes de résistance MLS$_B$ ($p> 0, 05$), à l'exception de l'acide fusidique qui était significativement plus élevé dans cMLS$_B$ que les autres phénotypes ($p=0,007$).

Conclusion: L'introduction de la détection de la résistance MLSB parmi les coques Gram-positifs dans la pratique microbiologique de routine peut jouer un rôle important dans la surveillance de la résistance inductible et ainsi prévenir l'échec du traitement.

Mots clés: *Staphylococcus aureus*; essai D; MLS$_B$ constitutif; MLS$_B$ inductible; phénotype SEP; la résistance

Introduction:

Macrolide, lincosamide, and strepto-gramin B (MLS$_B$) are a group of chemically distinct antibiotics that function primarily by inhibiting bacterial protein synthesis (1). The macrolides contain 14–16 membered lactone rings, the lincosamides are alkyl derivatives of proline that lack a lactone ring, and strepto-gramin B antibiotics are cyclic peptide com-pounds composed of two distinct factors (A and B) possessing synergistic inhibitory and bactericidal activity (2). MLS$_B$ antibiotics are clinically used as alternative drugs for the treatment of some *S. aureus* infections such as skin and soft tissue infections, especially in penicillin-allergic patients (3). However, wide-spread use of these antibiotics have selected for the development of resistant strains (4).

Resistance of staphylococci to erythro-mycin was first reported in 1956, a few years after its introduction (5). Bacterial resistance to macrolides occurs via at least three diffe-rent mechanisms including target modifica-tion, enzyme hydrolysis, and efflux pump (5, 6,7). The *erm* methylase gene mediates target modification by altering a site in the 23S rRNA, a common binding site for macrolides, lincosa-mides, and streptogramin B. Modification of the 23S rRNA confers cross-resistance to MLS$_B$ antibiotics. Hydrolytic enzymes such as eryth-romycin esterases (encoded by *ereA* and *ereB*) have been reported in *S. aureus* that lyses the lactone ring of the macrocyclic nucleus and

phosphotransferases, consequently with intro-ction of a phosphate on the 2'-hydroxyl group of the amino sugar. Macrolide efflux pumps which are ATP transporters (encoded by *msrA* and *msrB*) have also been reported in *S. aureus*. Expression of MLS$_B$ resistance in sta-phylococci may be constitutive or inducible. Constitutively resistant isolates are resistant to all macrolides, lincosamides, and strepto-gramin-B type antibiotics. Inducibly-resistant isolates, when tested individually, are only resistant to 14- and 15-membered macrolides, while 16-membered macrolides, commercially available lincosamide and streptogramin anti-biotics remain active (5).

Staphylococcus aureus is a clinically significant bacterial pathogen that causes a vast array of diseases in humans and animals alike. *S. aureus* diseases range from mild skin and soft tissues infections to severe and life-threatening infections such as septicaemia, toxic shock syndrome, endocarditis, and pneu-monia (8-9). Inducible clindamycin resistant *S. aureus* is a concern in clinical settings as they are not readily detected by routine labo-ratory methods. However, data on this anti-biotic cross-resistance among clinical isolates of Gram-positive cocci in Nigeria are inade-quate. Hence, this study was conducted to determine the prevalence of MLS$_B$ resistance among clinical isolates of *S. aureus* in the University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria.

Materials and method:

Study setting and ethical approval

University of Ilorin Teaching Hospital (UITH) is a tertiary healthcare centre located in Ilorin, Kwara State, North Central, Nigeria. The hospital renders its services to patients from various states including, Kwara, Kogi, Niger, Oyo, Osun, Ekiti, Lagos, and Kebbi, as well as the Federal Capital Territory (FCT) (10). Ethical approval for the study was obtained from the Ethical Review Board (ERB) of the UITH.

Study design

The study is a laboratory-based design that used clinical isolates of *S. aureus* recovered from clinical specimens submitted to the Department of Medical Microbiology and Parasitology of UITH.

Culture isolation and identification of *S. aureus*

Clinical specimens, including wound specimens, aspirates, eye swabs and ear swabs were inoculated directly on sheep blood and MacConkey agar plates. Bact/Alert-positive blood specimens were cultured on sheep blood, chocolate, and MacConkey agar plates. Inoculated plates were incubated aerobically while chocolate agar plates were incubated in microaerophilic environment in candle extinction jar. All culture plates were incubated at 37°C for 18-24 hours. Isolates on culture plates were identified morphologically by Gram's stain reaction and standard biochemical tests that included catalase, coagulase, DNase and mannitol fermentation tests. Isolates that were Gram-positive cocci in clusters, catalase-positive, coagulase-positive, DNase-positive, and mannitol-fermenters were identified as *S. aureus*.

Antibiotic sensitivity test (AST) of *S. aureus*

Antibiotic sensitivity testing (AST) was carried out on each *S. aureus* isolate using the modified Kirby-Bauer disc diffusion method. Bacterial inoculum was standardized to 0.5 McFarland standard before inoculating the surface of freshly prepared Mueller-Hinton agar (MHA) plates. The isolates were tested against the following antibiotics (Oxoid, UK); tetracycline (30µg), cotrimoxazole (1.25/23.75µg), mupirocin (5µg), linezolid (30µg), erythromycin (15µg), tigecycline (15µg), fusidic acid (10µg), fosfomycin (50µg), clindamycin (2µg), ciprofloxacin (5µg), rifampin (5µg), gentamicin (10µg) and cefoxitin (30 µg). *Staphylococcus aureus* ATCC 25923 was used as control strain for AST while *S. aureus* ATCC 43300 was used as control strain for cefoxitin disc test.

The diameters of zone of inhibition were measured with a calibrated ruler and interpretation of each isolate as sensitive, intermediate or resistant to the antibiotics was done using the Clinical and Laboratory Standards Institute (CLSI) breakpoints (11). Isolates with diameter of zone of inhibition ≤ 21 mm were classified as methicillin resistant (MRSA) and those with diameter ≥ 22 mm as methicillin sensitive (MSSA).

Phenotypic detection of MLS_B resistance

Freshly prepared Mueller-Hinton agar (MHA) plates were inoculated with standardized (0.5 McFarland) inoculum of the test organisms using a sterile cotton swab. Inducible clindamycin resistance was detected by placing erythromycin (15µg) and clindamycin (2µg) (Oxoid, UK) within 15-20 mm of each other, and incubating the plates aerobically at 37°C for 24 hours. The diameters of zone of inhibition were measured with a calibrated ruler and interpretation of the result of each isolate was done with the Clinical and Laboratory Standards Institute (CLSI) breakpoints (11).

Resistance of the test isolate to both erythromycin (zone diameter of inhibition ≤ 13mm) and clindamycin (zone diameter of inhibition ≤ 14 mm) discs was reported as constitutive resistance (cMLS_B), resistance to erythromycin alone with the formation of a D-shaped zone of inhibition between the two discs was reported as inducible resistance (iMLS_B; D-test positive), while resistance to erythromycin alone with no appearance of a D-zone was reported as MS phenotype (D-test negative) (11).

Statistical analysis

Statistical analysis was done using IBM SPSS version 21.0. Fisher exact test (with Odds ratio and 95% CI) was used to determine association between methicillin resistance and MLS_B resistance as well as between MLS_B resistance and antibiotic resistance. The Chi square test was used to measure significant difference between MLS_B resistance phenotypes and antibiotic resistance. P value less than 0.05 was considered to be statistically significant.

Results:

A total of 112 clinical isolates of *S. aureus* were recovered from clinical specimens. Of these, 31 (27.7%) were MLS_B-resistant. The prevalence of the MLS_B resistance phenotypes were MS phenotype (16.1%, n=18), cMLSB (6.2%, n=7), and iMLSB (5.4%, n=6). A total of 50 (44.6%) isolates were methicillin resistant (MRSA) while 62 (55.4%) were methicillin sensitive (MSSA) isolates (Table 1).

Table 1: Prevalence of MLS$_B$ resistance in MRSA compared to MSSA isolates

S. aureus strain	MLSB resistance n (%)	MLS$_B$ sensitive n (%)	Total n (%)	OR	95% CI	p-value
MRSA	22 (70.9)	28 (34.6)	50 (44.6)	4.627	1.88-11.388	0.000526*
MSSA	9 (29.1)	53 (65.4)	62 (55.4)			
Total	**31 (27.7)**	**81 (72.3)**	**112 (100)**			

MRSA = methicillin resistant *Staphylococcus aureus*; MSSA = methicillin sensitive *Staphylococcus aureus*; MLS$_B$ = macrolide-lincosamide-streptogramin B; OR=Odds ratio; CI = Confidence interval; n = number of isolates; *= statistically significant

Table 2: Univariate analysis of antibiotic resistance phenotypes of MLS$_B$-resistant *Staphylococcus aureus*

Antibiotics	cMLS$_B$ (%) (n=7)	iMLS$_B$ (%) (n=6)	MS phenotype (%) (n=18)	Total (%) (n=31)	X^2	p-value
Tetracycline	3 (42.9)	6 (100.0)	9 (50.0)	18 (58.1)	5.479	0.0646
Cotrimoxazole	3 (42.9)	1 (16.7)	7 (38.9)	11 (35.5)	1.185	0.5528
Ciprofloxacin	2 (28.6)	5 (83.3)	8 (44.4)	15 (48.4)	4.147	0.1258
Gentamicin	2 (28.6)	3 (50.0)	7 (38.9)	12 (38.7)	0.6259	0.7313
Fusidic acid	6 (85.7)	0	7 (38.9)	13 (41.9)	9.912	0.007*
Fosfomycin	2 (28.6)	2 (33.3)	5 (27.8)	9 (29.0)	0.06834	0.9664
Cefoxitin	5 (71.4)	3 (50.0)	14 (77.8)	22 (70.9)	1.686	0.4304

cMLS$_B$ = constitutive macrolide-lincosamide-streptogramin B; iMLS$_B$ = inducible macrolide-lincosamide-streptogramin B; MS = macrolide sensitive; X^2 = Chi square; n = number of isolates; * = statistically significant

Table 3: Univariate analysis of antibiotic resistance of MLS$_B$-resistant and MLS$_B$-sensitive *Staphylococcus aureus*

Antibiotics	MLS$_B$-sensitive (%) (n=81)	MLS$_B$-resistant (%) (n=31)	Total (%) (n=112)	OR	95% CI	p-value
Tetracycline	57 (70.4)	18 (58.1)	75 (61.5)	1.715	0.7271-4.047	0.2631
Cotrimoxazole	38 (46.9)	11 (35.5)	49 (40.2)	1.607	0.6829-3.780	0.2961
Ciprofloxacin	32 (39.5)	15 (48.4)	47 (38.5)	0.6966	0.3026-1.603	0.4022
Gentamicin	37 (45.7)	12 (38.7)	49 (40.2)	1.331	0.5720-3.099	0.5314
Fusidic acid	18 (22.2)	13 (41.9)	31 (25.4)	0.3956	0.1632-0.9588	0.0369*
Fosfomycin	15 (18.5)	9 (29.0)	24 (19.7)	0.5556	0.2133-1.447	0.3027
Cefoxitin	28 (34.6)	22 (70.9)	50 (44.6)	4.627	1.88-11.388	0.000526*

MLS$_B$ = macrolide-lincosamide-streptogramin B; OR=Odds ratio; CI = Confidence interval; n = number of isolates; * = statistically significant

All the MLS$_B$-resistant and MLS$_B$-sensitive isolates of *S. aureus* were sensitive to linezolid, rifampicin, mupirocin, and tigecycline. The resistance rates of the MLS$_B$-resistant isolates (n=31) to other antibiotics are; tetracycline (58.1%), ciprofloxacin (48.4%), fusidic acid (41.9%), gentamicin (38.7%), cotrimoxazole (35.5%), fosfomycin (29.0%), and cefoxitin (70.9%) (Table 2). Comparatively, resistance rates of the MLS$_B$-sensitive isolates (n=81) to other antibiotics are; tetracycline (70.4%), ciprofloxacin (39.5%), fusidic acid (22.2%), gentamicin (45.7%), cotrimoxazole (46.9%), fosfomycin (18.5%) and cefoxitin (34.6%) (Table 3).

Discussion:

The rapid spread of antibiotic resistant strains of *S. aureus* has complicated treatment options for infections, especially in low- and middle-income countries. This seeming difficulty due to antibiotic resistance led to the prominence of clindamycin, a MLS$_B$ antibiotic, for the treatment of skin and soft tissue infections caused by *S. aureus* and also for treatment in penicillin-allergic patients. Although rapid evolution of clindamycin resistance has been attributed to the use and misuse of clindamycin, inappropriate use of erythromycin can induce cross-resistance to clindamycin and streptogramin B antibiotics since all three antibiotics classes have a similar binding site. Hence, the inability to detect this resistance phenotype can lead to misuse of clindamycin, and consequently treatment failure (12-13).

MLS$_B$ resistance rate in this study was 27.7% which is comparable to 27.85% and 28.7% reported in Ethiopia and Nepal, India respectively (14–15). In a similar study, Ifediora et al., (16) reported 58.9% prevalence rate of MLS$_B$-resistant *S. aureus* in Abia State, Nigeria. Kishk et al., (17) reported a prevalence of 54.54% in Egypt while Lupinacci et al., (18) reported 68% in Sao Paulo, Brazil. In similar studies in India, Adhikari et al., (19) reported a prevalence of 54.4% in Nepal while Kavitha (20) reported a prevalence of 40.9% in Kilpauk. Furthermore, Sarrou et al., (21) reported a prevalence of 40.1% in Central

Greece, Goudarzi et al., (22) reported a prevalence of 42.16% in Tehran, Iran, and Jajajreh et al., (23) reported 60.6% in Jordan. Although, the prevalence of MLS_B-resistant *S. aureus* in our study seems lower compared to other locations, it still remains a significant cause of worry, especially in clinical settings.

The current study reported a 5.4% prevalence of iMLS_B which is lower than prevalence rates reported in Egypt (13.64%) and Ethiopia (24.1%) (15, 18). The reported iMLS_B prevalence is also lower than 12.1% reported in a similar study carried out in Abia State, Nigeria (16). Similar studies in India have also reported higher prevalence rates including Nepal (11.48%, 15.2%) and Kilpauk (15.5%) (14, 19–20). In similar studies in Tehran, Iran, Khodabandeh et al., (23) and Goudarzi et al., (24) reported 22.9% and 14.2% iMLS_B prevalence respectively. Similar studies have also reported higher iMLS_B prevalence rates including Brazil (7.2%), Central Greece (11.48%) and Jordan (46.5%) (17, 21–22). The seemingly low prevalence of iMLS_B among *S. aureus* is however not a call to complacency, but a call to a higher level of attention in the prescription of macrolides so as to keep this resistance low. Ultimately, the true prevalence of iMLS_B among *S. aureus* is a function of accurate diagnosis, geographical variation, peculiar characteristics of the healthcare facility, and the population under study (23).

The current study reported a 6.2% prevalence of cMLS_B *S. aureus* that is higher than prevalence rates reported in Ethiopia (2.53%) and India (4.6%) (14–15). This prevalence rate is however lower than 27.5% reported in Abia State, Nigeria (16). Similar studies have reported higher prevalence rates in Iran (56.2% and 23%), Egypt (38.64%), Brazil (60.8%), India (29.25% and 13.1%), Greece (26.44%), and Jordan (11.3%) (17–24). Lower prevalence of cMLS_B *S. aureus* reported in this study can be attributed to the rational prescription and usage of macrolides, both within community and hospital settings which has not favoured the prominence of hyper-resistant strains and molecular types.

In our study, the 16.1% prevalence of MS phenotype of MLS_B resistance is comparable to prevalence rates in Iran (16.6%) and India (16.6%) (14, 24), but lower than 19.2% reported in Abia State, Nigeria (16). Similar studies have reported lower prevalence of the MS phenotype in Egypt (2.27%), Ethiopia (1.26%), India (13.7% and 12.3%), Greece (2.90%), Iran (4.9%), and Jordan (2.82%) (15, 18–23). Our study also reported significant association between MLS_B resistance and methicillin resistance in clinical isolates of *S. aureus.* The prevalence of MLS_B resistance was significantly higher among MRSA isolates than MSSA strains of *S. aureus*. This assertion is in tandem with the reports of Ifediora et al., (16)

in Abia State, Nigeria and Kavitha (20) in Kilpauk, India. Similar studies have also reported higher MLS_B resistance among MRSA strains than MSSA strains (14–15,17–19,22–23). Furthermore, MRSA isolates have been globally reported to be multidrug resistant, especially the nosocomial strains. Hence, the spread of MRSA in clinical settings should be monitored to help thwart the possible evolution of MLS_B resistant strains of *S. aureus*.

Our study also reported varying antibiotic resistance patterns among MLS_B-resistant isolates, which is similar to reports of other studies on *S. aureus* (15,23). MLS_B resistance was associated with high resistance to fusidic acid in the study, however, there was no association between MLS_B resistance and resistance to other tested antibiotics. All MLS_B-resistant and sensitive *S. aureus* isolates in our study were susceptible to linezolid, rifampicin, tigecycline and mupirocin, hence, these antibiotics can be employed in the treatment of *S. aureus* infections in this region. However, caution should be taken in the administration of these antibiotics as antibiotic pressure can select for spontaneous evolution of resistant strains.

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Microscopic observation drug susceptibility assay for MDR TB detection

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Detection of multi-drug resistant tuberculosis (MDR TB) using microscopic observation drug susceptibility (MODS) assay in Lagos State, southwest Nigeria

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Abstract:

Background: Nigeria has the second highest case of multi-drug resistant tuberculosis (MDR TB) in Africa, estimated at 29,000 in 2015. The laboratory diagnosis of MDR TB in Nigeria is currently done using GeneXpert assay that generates results in less than two hours but can detect resistance to only rifampicin and has high-technology requirements. The objective of this study is to detect MDR TB using Microscopic Observation Drug Susceptibility (MODS) assay in Lagos State, Nigeria.

Methodology: A total of 80 patients who were positive for TB by GeneXpert in three Directly Observed Treatment Short-course (DOTS) centres in Lagos were studied. Spot sputum samples were collected from each patient and transported on ice-packs to Lagos University Teaching Hospital (LUTH) DOTS laboratory for decontamination. Culture and drug sensitivity test (DST) were performed on the pellets obtained by MODS assay in 24-well plates and examined with an inverted light microscope within 6 to 21 days of incubation at 35°C.

Results: Of the 80 patients, males were 43 (53.8%) while females were 37 (46.2%), with mean age of 36.2±11.6 years. Seventy-six (95.0%) of the patients had cough at presentation, 60 (75.0%) had not commenced anti-TB treatment, 15 (18.8%) were previously treated (PT) TB cases, and 14 (17.5%) were HIV positive. MODS assay detected *Mycobacterium tuberculosis* (MTB) in 52 (65.0%) patients across all the age groups but association between age groups and MTB detection by MODS assay was not significant (p=0.447). MODS assay detected MTB in 50 (83.3%) of 60 patients who had not commenced anti-TB drugs compared to 2 (10.0%) of 20 who had commenced anti-TB drugs at the time of sample collection (p<0.0001). Nine (60.0%) of 15 PT TB cases had MTB detected compared to 43 (66.2%) of 65 new cases of TB (p=0.7657). Nine of the 14 (64.3%) HIV positive patients were co-infected with MTB detected by MODS assay compared to 43 (65.2%) of 66 HIV negative patients (p=1.000). MDR TB was detected by MODS assay in 2 (2.5%) of the 80 patients (aged 30 and 38 years) who were previously identified as rifampicin resistant by GeneXpert assay (p=0.0003). The 2 MDR-TB cases were seen in the 15 PT (13.3%) cases, and in 1 of the 14 HIV (7.1%) positive patients.

Conclusion: MODS assay detected MDR-TB among PT TB patients in Lagos, Nigeria at the rate of 2.5%. Hence, MODS assay is an effective, low-tech, liquid culture technique to accurately detect TB and MDR-TB simultaneously.

Keywords: microscopic observation drug susceptibility; multi-drug resistant tuberculosis; directly observed treatment short-course; GeneXpert; Lagos

Détection de la tuberculose multirésistante (TB-MDR) à l'aide d'un test d'observation microscopique de sensibilité aux médicaments (MODS) dans l'État de Lagos, au sud-ouest du Nigeria

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Résumé:

Contexte: Le Nigéria compte le deuxième cas le plus élevé de tuberculose multirésistante (TB-MDR) en Afrique, estimé à 29 000 en 2015. Le diagnostic en laboratoire de la TB-MDR au Nigeria est actuellement effectué à l'aide du test GeneXpert qui génère des résultats en moins de deux heures, mais peut détecter la résistance à la seule rifampicine et a des exigences de haute technologie. L'objectif de cette étude est de détecter la tuberculose multirésistante à l'aide du test d'observation microscopique de la sensibilité aux médicaments (MODS) dans l'État de Lagos, au Nigeria.

Méthodologie: Un total de 80 patients positifs pour la tuberculose par GeneXpert dans trois centres de traitement de courte durée sous surveillance directe (DOTS) à Lagos ont été étudiés. Des échantillons ponctuels d'expectorations ont été prélevés sur chaque patient et transportés sur des sacs de glace au laboratoire DOTS de l'hôpital universitaire de Lagos (LUTH) pour décontamination. La culture et le test de sensibilité aux médicaments (DST) ont été effectués sur les culots obtenus par dosage MODS dans des plaques à 24 puits et examinés au microscope optique inversé dans les 6 à 21 jours d'incubation à 35°C.

Résultats: Sur les 80 patients, les hommes étaient au nombre de 43 (53,8%) tandis que les femmes étaient au nombre de 37 (46,2 %), avec un âge moyen de 36,2±11,6 ans. Soixante-seize (95,0%) des patients avaient toussé au moment de la présentation, 60 (75,0%) n'avaient pas commencé de traitement antituberculeux, 15 (18,8%) étaient des cas de tuberculose précédemment traités (PT) et 14 (17,5%) étaient infectés par le VIH. positif. Le test MODS a détecté *Mycobacterium tuberculosis* (MTB) chez 52 (65,0%) patients dans tous les groupes d'âge, mais l'association entre les groupes d'âge et la détection de MTB par le test MODS n'était pas significative (*p*=0,447). Le test MODS a détecté MTB chez 50 (83,3%) des 60 patients qui n'avaient pas commencé les médicaments antituberculeux par rapport à 2 (10,0%) des 20 qui avaient commencé les médicaments antituberculeux au moment du prélèvement de l'échantillon (*p*<0,0001). Neuf (60,0%) des 15 cas de TB PT avaient une MTB détectée contre 43 (66,2%) des 65 nouveaux cas de TB (*p*=0,7657). Neuf des 14 (64,3%) patients séropositifs pour le VIH étaient co-infectés par le MTB détecté par le test MODS, contre 43 (65,2%) des 66 patients séronégatifs pour le VIH (*p*=1 000). La tuberculose multirésistante a été détectée par le test MODS chez 2 (2,5%) des 80 patients (âgés de 30 et 38 ans) précédemment identifiés comme résistants à la rifampicine par le test GeneXpert (*p*=0,0003). Les 2 cas de TB-MR ont été observés chez les 15 cas PT (13,3%) et chez 1 des 14 patients séropositifs (7,1%).

Conclusion: Le test MODS a détecté la TB-MR chez les patients PT TB à Lagos, au Nigeria, à un taux de 2,5 %. Par conséquent, le test MODS est une technique de culture liquide efficace et de faible technologie pour détecter simultanément avec précision la tuberculose et la tuberculose multirésistante.

Mots-clés: observation microscopique de la sensibilité aux médicaments; tuberculose multirésistante; traitement de courte durée sous observation directe; GeneXpert; Lagos

Introduction:

Tuberculosis (TB) is an infectious disease caused by members of the *Mycobacterium tuberculosis* complex (MTBc) including *Mycobacterium bovis* (zoonotic tuberculosis) (1). TB is one of the biggest killers amongst infectious diseases despite the worldwide use of the live-attenuated Bacillus Calmette–Guérin (BCG) vaccine and several available antibiotics (2,3). Overall, 5–15% of estimated 2–3 billion people infected with *M. tuberculosis* will develop TB disease during their lifetime. However, immuno compromised individuals are more likely to develop TB (4). The emergence of various types of drug-resistant tuberculosis (DR-TB) has long been reported and this has complicated the control of TB worldwide (4,5,6).

Multi-drug resistant tuberculosis (MDR) is defined as resistance to isoniazid (INH) and rifampicin (RIF). It could result from either primary infection with a drug-resistant TB strain (primary resistance) or may develop during a patient's treatment due to inadequate therapy that enables the selection of drug resistance (7). High burden of TB and MDR-TB has been reported in countries with high populations.

Nigeria is estimated to have a population of 182 million with more than half its people currently below 30 years of age (8). Lagos, a port city is the most populous city in Nigeria with an estimated population of 21 million in 2016 (9). In 2016, Nigeria ranked 10th among the 30-high burden MDR-TB countries in the world and second among high burden MDR-TB countries in Africa, with 1,241 laboratory-confirmed MDR-TB cases of the esti-

mated 3,700-5,700 cases notified to National Tuberculosis and Leprosy Control Programme (4). Since the emergence of DR-TB, the conventional method was used for its diagnosis. However, with the advancement in technology, many new methods and techniques have been developed.

GeneXpert test, microscopic observation drug susceptibility (MODS) assay, liquid-based automated assay (MGIT) and line probe assay (LPA) have been endorsed by the World Health Organization (WHO) for detecting MDR-TB (10,11). However, in lower-middle-income settings such as Nigeria, the GeneXpert test is the diagnostic tool used for detecting MDR-TB. This test gives results in less than 2 hours, detects simultaneously both MTB and rifampicin resistance, and is free of cost for patients, although it is funded by international partners with counterpart-funding from the government of Nigeria. However, the GeneXpert test cannot detect INH resistance, which requires high tech nology. Moreover, in Lagos, the diagnosis of MDR TB still poses a challenge as unpublished data revealed that the operationalization of GeneXpert machines for testing MDR-TB is below optimization due to issues of interrupted electricity supply and inadequacy of sustainable air-conditioned ambience. In addition, as at the period of this study, there is a dearth of published data on the actual case detection rates of MDR-TB in Lagos State, Nigeria. Finally, the WHO recommends that GeneXpert test results should be confirmed with conventional drug susceptibility test (DST) (4), but this is rarely practiced in Lagos probably due to the long turn-around-time of conventional indirect DST.

The objective of this study therefore is to detect MDR-TB using microscopic-observation-drug-susceptibility assay among pulmonary TB patients previously positive for TB by the GeneXpert test in Lagos State, southwest Nigeria. The results from this study will provide baseline information to guide the tuberculosis control programme in the effective diagnosis and control of MDR-TB.

Materials and method:

Study settings and population
This study focused on pulmonary TB patients attending the three DOTS centres in Lagos for TB diagnosis, treatment and care between March and June 2017. The centres were Lagos University Teaching Hospital (LUTH) Idi-Araba, Mainland Hospital Yaba, and Randle

General Hospital, Randle Surulere, Lagos, Nigeria. The Mainland Hospital Yaba specializes in infectious disease care and control, with isolation wards for different forms of TB cases including MDR-TB. LUTH is a tertiary level hospital while Randle General Hospital is a secondary level hospital.

Ethical clearance
Ethical approval was obtained from the Health & Ethics Research Committees of Lagos University Teaching Hospital and Lagos Health Service Commission. All institutional guidelines of ethics involving human experimentation in research were strictly complied with. Written informed consent and confidentiality were obtained from each subject participant.

Study design and sample size
This was a cross sectional study carried out among pulmonary TB patients who were previously positive by GeneXpert test and were attending the Directly Observed Treatment Short-course (DOTS) centres in Lagos State. The sample size for this study was calculated using the formula; $n = (Z^2_{(1-\alpha)/2} \times pq)/d^2$ where 'n' is the expected sample size, '$Z^2_{(1-\alpha)/2}$' is the standard normal score at 95% confidence interval with type 1 error (=1.96), 'p' is the prevalence of MDR TB from a previous study of 4.1% (12), d is the absolute error set at 0.05. This gives the calculated minimum acceptable sample size of 60, but an addition of 40% was made to allow for attrition, bringing the calculated sample size to 84.

Inclusion and exclusion criteria
Patients of both genders aged 15 years and above were considered in this study. Such patients have been previously confirmed to have pulmonary TB by the GeneXpert test (Cepheid, Sunnyvale, USA). Patients with extra-pulmonary TB and children were excluded from the study.

Collection of demographic and clinical data
Interviewer-administered questionnaire was used to obtain information from each patient on demography, site of enrolment, prior history of TB treatment to differentiate 'new' case from 'previously treated' case, clinical symptoms (such as deep productive cough lasting more than 3 months, chest pain, unexplainable weight loss, night sweat, fever, loss of appetite, hemoptysis and shortness of breath) present during the time of sample collection, number of co-inhabitants, HIV status, use of

anti-retroviral drugs, duration on TB treatment (if any) and close contact with persons known or suspected of having MDR-TB.

Sample collection and transport

Each patient was instructed to produce a spot sputum sample at a designated area into a properly labeled, wide-mouth, sterile, leak-proof container with a tight-fitting lid. Thereafter, the samples were transported on ice packs to the DOTS laboratory, LUTH, Idi-Araba for processing. However, when processing was delayed, the samples were refrigerated at 2-8ºC to limit the growth of oral normal flora (13).

Sample processing and MODS assay

The samples were processed in a batch of 15 each in class II biosafety cabinet (BSC II) and level two biosafety (BSL-2) practices were followed to standard. The two major stages of processing were decontamination of samples, and culture and drug susceptibility testing by MODS assay. Two milliliters of sputum sample were placed in a 15 ml centrifuge tube (but when less than 2 ml, it was made up to 2 ml with phosphate buffer). Then, 2 ml NaOH-NALC solution was added and the tube was tightly capped, vortexed for 20 seconds and inverted for NaOH-NALC solution to make contact with the entire interior surface of the tube and lid. The tube could stand for at least 15 minutes but prolonged by a few minutes for particularly viscous samples. Thereafter, the tube was filled to 14ml with phosphate buffer (pH 6.8) to neutralize alkali and terminate the decontamination process and then mixed well by inverting 4 times. The resultant mixture was centrifuged at 3000g for 15 minutes and the supernatant was carefully poured off into a liquid waste container with 10% sodium hypochlorite while the pellet was retained (14).

Each 24-well plate was properly labelled with patients' identifiers and the date of inoculation. Using micropipettes, 4 tips were carefully filled with 100 µl of 7H9-OADC (enriched culture medium) and antibiotic working solutions (Column 2 in antibiotic dilution plate). Then 100 µl aliquots were added to the respective wells of column 1 in the 24-well plates. This was repeated until all columns have received 100 µl of medium (drug-free wells) or antibiotic working solutions (including negative control column 3). Thereafter, 900 µl of each final sample suspension (7H9-OADC-PANTA-sample pellet) was placed into the respective wells of a single column. This was

repeated with additional samples until all columns of the plate, except Column 3, were filled (or until all samples were plated). 900 µl of 7H9-OADC-PANTA medium without sample was placed in each of the 4 wells of Column 3 of each sample plate (negative internal controls). The plate was closed with a tight-fitting rubber cover but when the rubber cover was not available, it was closed with its lid and placed in a sealable zip lock polythene bag, sealed (the bag was not opened again from this point onwards) and incubated at 35°C. To minimize the risk of cross-contamination, the positive controls were set up in a separate plate after all plates with samples had been sealed and incubated.

Each of the two properly labelled control strains (drug susceptible MTB, and RIF and INH resistant strains) was adjusted to 1.0 McFarland standards. Using micropipettes with tips, 5 µl of each 1.0 McFarland-control strain suspension was mixed with 5ml of 7H9-OADC medium to make the positive control suspension. 900 µl of each positive control suspension was placed in the 4 wells of a column on the separate positive control plate and the four 100 µl aliquots of medium and antibiotic working solutions were added as was done for samples. The plate was capped with lid and sealed in a zip-lock bag, incubated at 37°C with other plates processed the same day.

Plates were examined under an inverted light microscope initially with 10x objective and subsequently with 4x objective lens. Drug-free wells were examined from day 5 through day 21. Two or more colony forming units (>2 CFU) with characteristics serpentine structures in both drug-free wells indicated MTB detection while resistance was defined as mycobacterial growth of >2 CFU in drug-containing wells on the same day that both drug-free wells were positive. Hence, growth in both isoniazid- and rifampicin-containing wells indicated MDR. The result was defined as "indeterminate" if only 1 CFU appears in either drug-free well or in both while results were considered "valid" when the internal negative and positive control wells were examined and interpreted in like manner as the sample wells (13).

Statistical analysis

The Statistical Package for the Social Sciences (IBM SPSS For Windows version 23) was used to analyze data. Qualitative variables, proportions and associations were compared using Chi-square test. All reported confidence intervals were two-sided 95% confidence inter-

vals and *p* < 0.05 was regarded as statistically significant.

Results:

Of the 80 patients previously diagnosed with pulmonary TB by GeneXpert assay whose spot sputum samples were collected, 37 (46.2%) and 43 (53.8%) were females and males respectively, with a mean age of 36.2± 11.6 years. Of these patients, 78 (97.5%) were positive for MTB and 2 (2.5%) for rifampicin-resistant MTB. At the time of sample collection, 20 (25.0%) patients had commenced anti-TB drugs for less than four weeks while 60 (75.0%) had not commenced anti-TB drugs. Fifteen (18.8%) were PT TB cases while 65 (81.2%) were 'new' cases of TB. Five (6.3%) had previous contact with MDR-TB patients while 72 (90%) did not have any contact with MDR-TB patients, and 3 (3.7%) were unsure of previous contact with any MDR-TB patient. Fourteen (17.5%) patients were HIV positive while 66 (82.5%) were HIV negative (Table 1).

MODS assay detected MTB in 52 (65.0%) of the 80 patients across all the age groups, and 9 (17.3%) of these were co-infected with HIV but the association between MTB detection and age groups (*p*=0.447), and co-infection of MTB with HIV (*p*=1.000) were not statistically significant (Fig 1 and Table 2). MODS assay detected MTB in 50 of 60 (83.3%) patients who were not on anti-TB drugs compared to 2 (10.0%) of 20 who were on anti-TB drugs at the time of sample collection (*p*< 0.0001) (Table 2). MODS assay detected MTB in 9 of 15 (60.0%) PT TB cases compared to 43 (66.2%) of 65 'new' cases of TB (*p*=0.7657).

MODS assay detected MDR-MTB (i. e. resistant to both rifampicin and isoniazid) in only 2 (2.5%) of the 80 patients (aged 30 and 38 years) and these occurred in 2 of the 15 (13.3%) PT cases, and in 1 of the 14 (7.1%) HIV positive patients. The 2 MDR TB cases detected by the MODS assay were the 2 cases of rifampicin resistance (RR) previously detected by GeneXpert assay, while 78 patients that were not previously RR were not MDR TB positive by the MODS assay (*p*=0.0003) (Table 2).
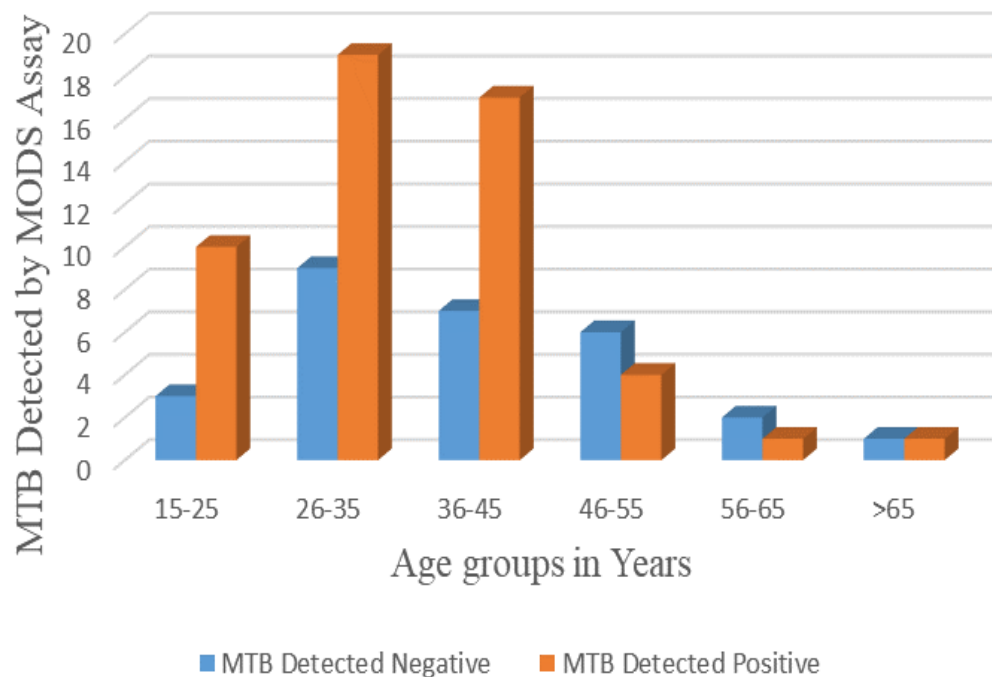

Fig 1: Frequency distribution of *Mycobacterium tuberculosis* (MTB) cases detected by MODS assay with respect to age groups (χ^2 = 5.793, *p* = 0.447)

Table 1: Demographic and clinical characteristics of the patients from three DOTS centers in Lagos, Nigeria

Characteristics	Frequency (%)
Gender	
Female	37 (46.2)
Male	43 (53.8)
Age group (years)	
15-25	13 (16.3)
26-35	28 (35.0)
36-45	24 (30.0)
46-55	10 (12.5)
56-65	3 (3.8)
> 65	2 (2.5)
Mean age (years)	36.2 ± 11.6
Occupation	
Self-employed	58 (72.5)
Student	9 (11.3)
Civil servant	6 (7.5)
Others	7 (8.7)
DOTS Centre	
LUTH	12 (15.0)
Randle General Hospital	31 (38.8)
Mainland Hospital Yaba	37 (46.2)
HIV status	
Positive	14 (17.5)
Negative	66 (82.5)
Previous history of TB	
Yes	15 (18.8)
No	65 (81.2)
Previous contact with MDR-TB patients	
Yes	5 (6.3)
No	72 (90.0)
Not sure	3 (3.7)
TB symptoms present at screening	
Cough	76 (95.0)
Fever	29 (36.3)
Night sweats	48 (60.0)
Weight loss	66 (82.5)
Chest pain	29 (36.3)
Hemoptysis	11 (13.8)
Currently on TB treatment	
Yes	20 (25.0)
No	60 (75.0)
Previously treated TB cases	
Yes	15 (18.8)
No	65 (81.2)

LUTH = Lagos University Teaching Hospital; DOTS=Directly Observed Therapy Short-course; MDRTB = Multi-Drug Resistant Tuberculosis;
TB = Tuberculosis; HIV=Human Immunodeficiency Virus

Table 2: Bivariate analysis of variables associated with MTB detection by MODS assay

Variables	Outcome	MTB detected	MTB not detected	OR	95% CI	*p* value
HIV status	Negative	43 (65.2)	23 (34.8)	1.039	0.3113-3.466	1.000
	Positive	9 (64.3)	5 (35.7)			
TB treatment at time of sample collection	No	50 (83.3)	10 (16.7)	45.000	8.983-225.43	<0.0001
	Yes	2 (10.0)	18 (90.0)			
Previously treated TB	No	43 (66.2)	22 (33.8)	1.303	0.4109-4.132	0.7657
	Yes	9 (60.0)	6 (40.0)			
Rifampicin resistant	No	0	78 (100)	0.001274	2.073×10^{-5} -7.828×10^{-2}	0.0003
	Yes	2 (100)	0			

MTB=*Mycobacterium tuberculosis*; HIV=Human Immunodeficiency Virus; TB=Tuberculosis; OR=Odds Ratio; CI = Confidence Interval

Discussion:

The findings of this study revealed that MTB was detected by MODS assay in 52 of 80 (65.0%) patients' samples previously diagnosed positive by GeneXpert assay. Of the 28 samples negative by MODS assay, 18 (64.3%) were already on anti-TB drugs while the remaining 10 (35.7%) could have been negative probably due to poor quality of samples, which has been reported to affect culture yield in previous studies (15-18). Unfortunately, in this study, the quality of samples was not assessed. Of the 20 patients who were on anti-TB drugs at the time of sample collection, 2 (10.0%) were positive for MTB by MODS assay and simultaneously resistant to isoniazid and rifampicin, indicating failure of treatment. This agrees with 10.0% treatment failure rates by MODS assay reported in Zimbabwe, although these were among patients who had been on treatment for more than one month (19), but higher than the failure rate of 2.5% reported by Alobu et al., (20) by smear microscopy among patients who have been on treatment for five months.

Interestingly, the 2 MDR-TB cases detected by MODS in this study were previously diagnosed to be rifampicin-resistant by Gene-Xpert test. The findings of our study show that all RR MTB are MDR, which is consistent with the WHO report (4) that RR in the absence of INH resistance is very uncommon. Furthermore, our study reveals that the 2.5% detection rate of MDR-TB by MODS assay is lower compared to 18.0% in South Africa (21) with high HIV-prevalent settings, 12.3% of DR-TB suspects in Harare, Zimbabwe (22), 8.8% of smear-positive TB patients in Addis Ababa, Ethiopia (23), and 4.1% of smear-positive PTB patients in Nigeria (12). The higher case detection in these other studies may be attributed to the larger sample size involved. Also, a study conducted in India reported 20% MDR-TB case detection by MODS assay among smear-positive samples. This is higher than 2.5% reported in our study as India is known for its large population having the second highest prevalence of MDR-TB in the world (4). Another study by Minh Ha et al., (24) which evaluated MODS assay among new TB suspects in Vietnam reported case detection of MDR-TB to be 2.7%, which is similar to the findings of 2.5% rate reported in our study.

Among the previously treated cases, there was a 13.3% case detection rate of MDR-TB by MODS assay in our study, which is higher than the estimated 2.5% MDR-TB rates among previously treated TB cases in Nigeria (4). The higher detection rate may mean the prevalence of MDR-TB has indeed increased in this study population and can be attributed to non-compliance to and inadequate treatment. Such patients pose a great threat to public health as they may transmit these MDR strains to other people and thus, hamper the control of TB and MDR-TB. In addition, this high rate may indicate that there is a drastic increase in MDR-TB among previously treated TB cases in Nigeria between 2016 and 2017.

Lastly, in relation to HIV status, one of the two MDR-TB cases reported was HIV positive, which constituted 7.1% (1/14) of the HIV positive patients, while the other MDR-TB case was HIV negative, which formed 1.5% (1/66) of HIV-negative patients in the study, but there was no significant difference in the finding (OR = 5.00, 95% CI = 0.2934 - 85.216, p = 0.3212), indicating no association between HIV and MDR-TB. Similar findings have been reported in Jos and Kwara in north-central Nigeria (25,26), and Calabar in south-south Nigeria (27). However, the findings of our study should be interpreted with caution due to the small sample size because, in contrast, other studies have found a significant association between HIV status and DR-TB (28,29).

Conclusion:

MODS assay detected MDR-TB among previously treated TB patients in Lagos State at the rate of 2.5%. Hence, MODS assay is an effective, low-tech, liquid culture technique to accurately detect tuberculosis and multidrug-resistant tuberculosis simultaneously in Lagos, Nigeria.

Acknowledgements:

Conflict of interest:

Authors declare no conflict of interest

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Original Article   **Open Access**

Comparative analysis of haematological parameters in HIV patients with co-infections of hepatitis B & C, and HIV-negative patients in Rivers State, Nigeria

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Abstract:

Background: Human immunodeficiency virus (HIV) has continued to be a threat to global health with several deaths recorded despite the introduction of highly active antiretroviral therapy (HAART). Co-infection of hepatitis B and C is now one of the leading causes of death among HIV-infected patients due to some haematological abnormalities and immunological impairment. This study was conducted to compare some haematological parameters of HIV-infected patients with hepatitis B and C co-infections from three hospitals in Rivers State, Nigeria
Methodology: This was a comparative cross-sectional study of randomly recruited HIV-patients from antiretroviral therapy (ART) clinic and HIV-negative patients from medical out-patient department (MOPD) of three different hospitals in Rivers State, Nigeria. Socio-demographic information of each participant was obtained with a structured questionnaire. Four millilitres of blood were collected from each participant by venipuncture; 2 ml each were dispensed into ethylene diamine tetra acetic acid (EDTA) and plain bottles for estimation of full blood count (FBC), cluster of differentiation 4 (CD4), HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) serology.
Results: A total of 375 participants (M:F ratio 1:1.5, age range 10-69 years) comprising 150 HIV patients on ART, 135 ART-naive HIV patients, and 90 HIV-negative patients (control) were recruited. Comparison of haematological parameters among HIV-negative (control), HIV-infected, and HIV/HBV/HCV, HIV/HCV and HIV/HBV co-infected patients showed significant increase ($p<0.05$) in mean lymphocyte count (%) of 36.69±13.25, 42.02±12.75, 46.53±8.36, 47.64±14.35, and 49.61±5.81, and a significant decrease ($p<0.05$) in mean neutrophil count (%) of 54.43±13.52, 46.33±13.04, 44.23±9.30, 41.66±12.94 and 40.86±7.56 respectively. The mean platelet count (10^9/L) in HIV-negative control, HIV-infected, and HIV/HCV, HIV/HBV and HIV/HBV/HCV co-infected patients showed significant decrease ($p<0.05$) of 235.25±109.52, 229.26±104.70, 152.25±56.64, 138.69±56.25, and 130.33± 79.51, as well as a significant decrease in CD4 cell counts (cells/μl) of 803.40±211.24, 619.67±334.13, 590.63± 312.20, 550.15±311.72, and 406.49±261.75 respectively.
Conclusion: Alterations in the haematological parameters can lead to serious complications in HIV individuals co-infected with HBV and/or HCV. Therefore, HBV and HCV screening for every HIV-infected patient should be made mandatory in Nigeria

Keywords: antiretroviral; co-infection; hepatitis B virus; hepatitis C virus; human immunodeficiency virus

Analyse comparative des paramètres hématologiques chez les patients séropositifs co-infectés par les hépatites B et C et chez les patients séronégatifs dans l'État de Rivers, au Nigeria

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Résumé:

Contexte: Le virus de l'immunodéficience humaine (VIH) a continué d'être une menace pour la santé mondiale avec plusieurs décès enregistrés malgré l'introduction de la thérapie antirétrovirale hautement active (HAART). La co-infection par l'hépatite B et l'hépatite C est aujourd'hui l'une des principales causes de décès chez les patients infectés par le VIH en raison de certaines anomalies hématologiques et d'une déficience immunologique. Cette étude a été menée pour comparer certains paramètres hématologiques de patients infectés par le VIH avec des co-infections par l'hépatite B et C dans trois hôpitaux de l'État de Rivers, au Nigéria.

Méthodologie: Il s'agissait d'une étude transversale comparative de patients séropositifs recrutés au hasard dans une clinique de traitement antirétroviral (TAR) et de patients séronégatifs du service médical ambulatoire (MOPD) de trois hôpitaux différents dans l'État de Rivers, au Nigeria. Les informations socio-démographiques de chaque participant ont été obtenues à l'aide d'un questionnaire structuré. Quatre millilitres de sang ont été prélevés sur chaque participant par ponction veineuse; 2 ml chacun ont été distribués dans des flacons d'acide éthylène diamine tétra acétique (EDTA) et simples pour l'estimation de la formule sanguine complète (FBC), du groupe de différenciation 4 (CD4), du VIH, du virus de l'hépatite B (VHB) et du virus de l'hépatite C (VHC) sérologie.

Résultats: Un total de 375 participants (rapport M: F 1: 1,5; tranche d'âge de 10 à 69 ans) comprenant 150 patients séropositifs sous TAR, 135 patients séropositifs naïfs de TAR et 90 séronégatifs (contrôle) ont été recrutés. La comparaison des paramètres hématologiques chez les patients séronégatifs pour le VIH (contrôle), infectés par le VIH et co-infectés par le VIH/VHB/VHC, le VIH/VHC et le VIH/VHB a montré une augmentation significative ($p<0,05$) du nombre moyen de lymphocytes (%) des 36,69±13,25, 42,02±12,75, 46,53±8,36, 47,64±14,35 et 49,61±5,81, et une diminution significative ($p<0,05$) du nombre moyen de neutrophiles (%) de 54,43±13,52, 46,33±13,04, 44,23±9,30, 41,66±12,94 et 40,86±7,56 respectivement. La numération plaquettaire moyenne (10^9/L) chez les patients témoins séronégatifs, infectés par le VIH et co-infectés par le VIH/VHC, le VIH/VHB et le VIH/VHB/VHC a montré une diminution significative ($p<0,05$) de 235,25±109,52, 229,26±104,70, 152,25±56,64, 138,69±56,25 et 130,33±79,51, ainsi qu'une diminution significative du nombre de cellules CD4 (cellules/μl) de 803,40±211,24, 619,67±334,13, 590,63±312 550.15±311,72 et 406,49±261,75 respectivement.

Conclusion: Des altérations des paramètres hématologiques peuvent entraîner de graves complications chez les personnes VIH co-infectées par le VHB et/ou le VHC. Par conséquent, le dépistage du VHB et du VHC pour chaque patient infecté par le VIH devrait être rendu obligatoire au Nigeria

Mots clés: antirétroviral; co-infection; Virus de l'hépatite B; virus de l'hépatite C; virus de l'immunodéficience humaine

Introduction:

Human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) emerged some decades ago to become a global pandemic especially in the sub- Saharan Africa where it has brought so much pain and hardship to many people. Non-adherence to antiretroviral therapy (ART) and delay in administering ART has resulted in reduction of T-cells in patients with HIV infection. The immuno-suppression experienced by HIV infected individuals renders them very susceptible to opportunistic infections which could be viral, bacterial, parasitic or fungal. Co-infection with HBV and/or HCV is now known to be caused by drug resistance among individuals with related haematological abnormalities (1,2).

Globally, HBV and HCV infections remain major causes of public health problems and are responsible for high morbidity and mortality rate. They continue to pose very serious clinical issues in developing countries because majority of the populace are not even aware of these infections and the task of planning for the screening of the general populace is not an easy one. In most cases, detection is done very late and most of those infected are not able to pay for the high cost of effective management (3,4). The World Health Organisation (WHO) report reports that 2 - 4% and 4 - 5% of the global population of HIV positive patients infected with HBV and HCV respectively (2). In developing countries, the routes of transmission of these viruses and risk factors are similar and include; transfusion of infected blood and blood products, sexual contact, sharing of injection needles by drug addicts, using unsterilized sharp objects during surgical operation and vertical transmission from mother to child (5,6,7).

HIV-infected patients are known to have different haematological challenges including, anaemia, thrombocytopenia, lymphopenia and neutropenia (8). HIV disease causes alteration in these parameters as the disease progresses and can occur among those patients on anti-retroviral therapy (ART) and those who are ART-naïve (9). An early examination of these parameters will help the clinicians in better management of HIV patients.

Anaemia is a common feature in HIV patients with a rate of 13- 95%, the commonest form being, normocytic normochromic anaemia followed by microcytic anaemia (10,11). Thrombocytopenia is another haematological abnormality observed among about 3-40% of HIV patients and tends to appear in all stages of the infection. Major causes could be platelet reduction due to auto immunity by cytokines, cross-reacting cytokines which are set against HIV proteins, mostly the gp120 and p24. This type of alteration is known as immune thrombocytopenic purpura (ITP) which involves depletion in values of platelet with regular values of haematocrit and white blood cell counts (9).

Another leucopenia normally observed among these subjects is neutropenia which affects about 10-30% of them at the final stage of the infection. Leucopenia could also be caused by co-infections such as, tuberculosis, hepatitis, histoplasmosis, leishmaniasis and others. Co-infection of HBV and/or HCV with HIV affects the bone marrow in a way that the granulocyte macrophage colony-stimulating factor (GM-CSF) which is responsible for haematopoiesis of the white blood cells in stromal with a resultant effect on the granulocyte-macrophage pedigree, are depleted, resulting in leukopenia and neutropenia. Also, HIV may cause lymphopenia with as the disease progresses, with resultant depletion of CD4^{+} T-lymphocytes (9,10). Thus, the objective of this comparative study is to describe the haematological changes that could occur in HIV patients co-infected with HBV/HCV and HIV-negative controls, in order to assist in preparing better treatment plan for these patients.

Materials and method:

Study area
This study was carried out in three different hospitals within the geopolitical zones in Rivers State, Nigeria; Zonal Hospital Ahoada (Rivers West), Zonal Hospital Bori (Rivers South East) and Rivers State University Teaching Hospital (Rivers East).

Study design and participants
This was a cross-sectional study of 375 participants consisting of 285 HIV-infected patients (150 on antiretroviral therapy and 135 ART-naive) and 90 HIV-negative patients. The HIV-patients (of both gender and different age groups) were systematically recruited from the ART clinics of the three hospitals (n=95 from each hospital) and the HIV-negative patients from medical out-patient department (MOPD) of the three hospitals (n=30 from each hospital).

Ethical approval and consent
Ethical approval for the study was obtained from the office of the Permanent Secretary of the Rivers State Ministry of Health and the Rivers State Hospital Management Board. The consent of each participant was obtained through the filling of the consent form.

Calculation of sample size
The minimum sample size required for the study was calculated using the formula; $N=Z^2pq/d^2$ where, N=sample size, Z=statistic corresponding to level of 95% confidence level (1.96), p=expected prevalence of 3.8% or 0.038 (12), d=level of significance (allowable error) which is 5% (0.05) and q=1-p. This gives a minimum sample size of 56, which was increased to 375.

Sample collection and preparation
The socio-demographic information of each participant was obtained using a structured questionnaire. Four millilitres of venous blood were collected from each participant and 2 ml each dispensed into ethylene diamine tetra acetic acid (EDTA) and plain bottles for haematological and serological analysis. Separation of samples in plain bottles was in a centrifuge at 1500 rev/min for 5 minutes. Storage of serum and whole blood was done at -20°C and 2-8°C respectively for samples that were not analysed the same day.

Determination of full blood count
Full blood count (FBC) was determined using Sysmex XP-300 machine for total white blood count (tWBC), red blood cells (RBC), haemoglobin concentrations (HB), packed cell volume (PCV), neutrophils (NEUT), lymphocytes (LYM), monocytes, eosinophils and basophils (MEB), and platelets (PLT).

Briefly, whole blood was allowed to mix for 10 minutes on the blood mixer. The power switch was turned on, self-check, auto rinse and background check were automatically performed and the "ready" (ready for analysis) appeared. The test and control samples were introduced into the instrument through the probe. Quality control check was done on the sample with the control blood provided (Eight check-3WP) using X control or L-J control program from the manual. Whole blood mode was selected (as the case may be) and samples numbers imputed. The plug was removed from the vacutainer tube while preventing the scattering of blood. Sample or control was introduced through the sample probe and the start button pressed for the sample or control to be aspirated. The sample tube was removed when

the buzzer made a "beep" sound and when the LCD screen displayed "Analyzing". The instrument automatically executed the analysis and displayed the result on the LCD screen. After this, the unit turns to the "Ready" status becoming ready for analysis of the next sample. On the ready status the shut-down key was pressed after each day's work. Shut down screen appeared. The machine prompted for the rinse (cell clean) solution, the cell clean solution was set to the sample probe and the start button was pressed holding the cell clean in the same state. The buzzer made the "beep" sound which indicated the completion of aspiration. The cell clean solution was removed from the sample probe and shutdown executed automatically.

Determination of CD4 cell count

CD4 cells count was determined using the BD FACS Count machine (BD Biosciences, USA) which uses the principle of flowcytometry (13,14). Reagent tubes were labeled, vortexed for 5 seconds and opened using the coring station. Test samples were properly mixed and 50 µl of whole blood was pipetted and added to the reagent tube. The tubes were capped, mixed and incubated for 60 mins in the dark at 20-25°C. The tube was later uncapped and 50 µl of fixative was added to the tubes and recapped. The 'on button' was switched on, daily cleaning carried out, control code verified and control samples were run. The 'sample button' on the machine was pressed, verification of the reagent code was done and sample ID entered. The CD4 tubes were vortexed again, uncapped, placed in the sample holder and the 'run button' pressed. After each analysis, the result was displayed automatically on the screen and the tube was removed.

HIV screening

The National algorithm for HIV screening in Nigeria which involves the use of three test kits was employed, with two for parallel testing and one for a tie-breaker. The three kits were Determine-HIV 1/2 (Abbott Japan Co., Ltd., Germany), Uni-Gold-HIV 1/2 (Trinity Biotech, France) and Stat-Pak Dipstick (Chembio Diagnostic System Inc). The manufacturers' standard operating procedure (SOP) was followed in performance of the test. HIV sero-positivity was defined as a reactive result on two of the test kits. Non-reactive subjects were considered sero-negative.

Determine HIV 1/2 test principle and procedure

The Abbott Determine HIV 1/2 is an *in vitro* visually read, rapid immuno-chromatographic test for the qualitative detection of anti-

bodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. It involves removing the test device from the pouch and applying 50µl of the test sample to the test pad (marked by arrow symbol) and then waiting for the sample to migrate through the conjugate pad. To ensure assay validity, a procedural control is incorporated in the device and labeled "control". If the control bar does not turn red at the completion of the assay, the test result is invalid and the sample must be retested. The result was read within 15 minutes

HBV and HCV screening

The screening involved testing serum sample of each patient for the presence of hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus using 'DiaSpot', a commercially available test kits for the detection of HBsAg or anti–HCV antibody based on principle of sandwich immunoassay in which recombinant antigens are employed sufficiently to identify anti-HCV or HBsAg with high sensitivity and specificity.

Chromogen embedded moves by diffusion to develop colour at test band region where Ag-Ab-Ag complex is formed. Simultaneously, human immunoglobulins present in the serum will be captured by anti-human globulin antibodies at the control region on which the chromogen also impact colour to give a control band. The test device was removed from pouch and dipped into specimen for 3 seconds with the arrow pointing downwards. The device was later laid on a clean, dry and non-absorbent surface. The result was read within 15 minutes.

Quality control

The samples were tested on the machines after running the controls and the standard operational procedures (SOPs) were strictly followed to ensure accuracy of the results.

Statistical analysis

Data generated were analysed using the Statistical Package for Social Sciences (SPSS) and Excel (version 22.0) package. Data presented as mean±SD were compared using the Students' *t*-test while demographic data were compared using Chi square test, and *p* value < 0.05 was considered statistically significant.

Results:

A total of 375 participants from three hospitals; Rivers State University Teaching Hospital (RSUTH), Zonal Hospital Bori (ZHB) and Zonal Hospital Ahoada (ZHA) representing the

three senatorial districts in Rivers State, were recruited for the study (Table 1). One hundred and fifty (40.0%) were HIV-infected patients on ART, 135 (36.0%) were ART-naïve HIV-infected patients while 90 (24.0%) were HIV-negative controls. Male constituted 40.3% (151/375) while females constituted 59.7% (224/375) with a male to female ratio of 1: 1.5. The age groups most represented were 20-29 years (26.7%) and 30-39 years (23.7%), while the least represented age groups were 60-69 years (7.2%) and 10-19 years (8.3%).

Table 2 shows the haematological parameters of HIV-infected patients (who were on ART and who were ART-naïve). There was no significant difference ($p>0.05$) in the mean values of the total white blood cells, red blood cells, haemoglobin concentration and haematocrit between HIV patients (ART and ART-naive) and HIV-negative patients. On the other hand, there was significant increase ($p<0.05$) for MED (majorly, monocytes) and lymphocytes while the neutrophils and platelets showed significant decrease ($p<0.05$) in HIV patients (on ART and ART-naïve) when compared with HIV-negative patients.

The comparison of the mean±standard deviation (SD) for these parameters showed no significant difference ($p>0.05$) in mean values in mean values of all the haematological parameters when comparing HIV-infected males on ART with HIV- infected females on ART as shown in Table 3 but CD4 cell count was significantly higher in male than female ($p=0.007$).

Table 1: Demographic and clinical characteristics of HIV-infected and HIV-negative patients in three hospitals, Rivers State, Nigeria

Characteristics	No of subjects on ART (%) (n=150)	No of ART-naïve subjects (%) (n=135)	No of control subjects (%) (n=90)	Total no of subjects (%) (n=375)	p value
Gender					
Male	58 (38.6)	59 (43.7)	34 (37.8)	151 (40.3)	0.5902
Female	92 (61.3)	76 (52.3)	56 (62.2)	224 (59.7)	
Age group (years)					
10-19	12 (8.0)	11 (8.2)	8 (8.8)	31 (8.3)	0.9940
20-29	37 (24.6)	38 (28.2)	25 (27.7)	100 (26.7)	
30-39	38 (25.3)	29 (21.5)	22 (24.4)	89 (23.7)	
40-49	30 (20.0)	24 (17.8)	15 (16.6)	69 (18.4)	
50-59	21 (14.0)	23 (17.0)	15 (16.6)	59 (15.7)	
60-69	12 (8.0)	10 (7.4)	5 (5.5)	27 (7.2)	
Hospital					
RSUTH	50 (33.3)	45 (33.3)	30 (33.3)	125 (33.3)	1.0000
ZHB	50 (33.3)	45 (33.3)	30 (33.3)	125 (33.3)	
ZHA	50 (33.3)	45 (33.3)	30 (33.3)	125 (33.3)	

ART=Antiretroviral therapy; RSUTH = Rivers State University Teaching Hospital; ZHB = Zonal Hospital Bori; ZHA = Zonal Hospital Ahoada

Table 2: Haematological Parameters of HIV- infected patients and HIV-negative patients in the three hospitals, Rivers State, Nigeria

Haematological parameters	HIV-infected on ART (n=150)	HIV-infected ART-naive (n=135)	HIV-negative control (n=90)	F value	p value
WBC (x10^9/L)	5.69 ± 3.19	5.58±2.36	6.23±0.37	0.67	0.51
RBC (x10^{12}/µL)	3.97 ± 0.93	7.09±3.64	4.19±0.89	0.76	0.47
HB (g/dL)	10.92 ± 2.47	10.76±2.11	10.87±2.43	0.16	0.83
HCT (%)	33.88 ± 7.57	35.63±6.30	33.53±7.35	2.58	0.77
MEB (%)	11.06 ± 5.55[b]	11.03±4.77[b]	8.49±3.25[a]	21.01	<0.001*
LYM (%)	45.06 ±10.76[b]	41.77±13.46[b]	36.70±13.25[a]	16.78	<0.001*
NEUT (%)	44.07 ± 17[a]	46.77±13.35[a]	54.43±13.52[b]	9.01	<0.001*
PLT (x10^9/L)	211.11±70.62[b]	225.71±89.24[ab]	261.40±85.59[a]	4.45	0.012*

WBC= white blood cells; RBC=Red blood cell; HB=Haemoglobin; HCT=Haematocrit; MEB= Monocytes, Eosinophils, Basophils; LYM = Lymphocytes NEUT = Neutrophils; PLT= Platelets. Values with different subscripts are significantly different ($p<0.05$)

Table 3: Haematological parameters of HIV-infected patients on ART with respect to gender

Gender	WBC (x10^9/L)	RBC (x10^{12}/µL)	HB (g/dl)	PCV (%)	LYM (%)	MEB (%)	NEUT (%)	PLT (x10^9/L)	CD4 (cells/µl)
Male	5.49±2.59	3.91±0.80	11.15±2.6	34.59±7.8	46.37±10.3	11.76±6.2	42.48±10.9	231.52±82.1	729.62±244.64
Female	5.83±3.77	4.01±0.80	10.55±2.2	32.74±7.1	44.24±11.0	10.61±5.1	45.07±11.2	214.42±72.8	621.46±303.68
p value	0.53	0.14	0.32	0.59	0.63	0.23	0.78	0.42	0.007*
T value	0.4	2.22	0.01	0.3	0.24	1.48	0.08	0.66	7.37

WBC=White blood cell; RBC=Red blood cell; HB=Haemoglobin; MEB=Monocytes, Eosinophils, Basophils; LYM = Lymphocytes; NEUT = Neutrophils; PLT = Platelet, HCT = Haematocrit; * = statistically significant ($p<0.05$)

Table 4: Comparison (ANOVA) of haematological parameters in HIV co-infections and HIV-negative controls

Haematological parameters	HIV/HBV	HIV/HCV	HIV/HBV/HCV	HIV	Control	F-value	p value
WBC (x10^9/L)	5.08±1.69	4.28±1.08	4.43±1.24	5.69±2.33	6.23±2.37	1.67	1.50
RBC (x10^{12}/µL)	3.88±0.82	4.44±1.21	3.57±0.81	4.08±0.97	4.19±0.69	1.11	0.35
HB (g/dl)	10.99±2.56	10.87±2.43	9.17±2.64	10.98±2.11	11.53±2.67	0.65	0.63
HCT (%)	35.52±7.21	33.53±7.35	29.60±8.41	35.75±6.19	36.21±8.07	1.37	0.25
LYM (%)	47.64±14.35[c]	49.61±5.81[d]	46.53±8.36[c]	42.02±12.75[b]	36.69±13.25[a]	2.76	<0.001*
MEB (%)	10.85±5.25	9.53±3.74	9.23±4.03	11.31±5.10	8.84±3.08	1.91	0.11
NEUT (%)	41.66±12.94[a]	40.86±7.56[a]	44.23±9.30[b]	46.33±13.04[c]	54.43±13.52[d]	3.66	0.007*
PLT (x10^9/L)	138.69±56.25[c]	152.25±56.64[b]	130.33±79.51[a]	229.26±104.70[d]	235.25±109.52[e]	5.02	0.001*
CD4 (cells/µl)	550.15±311.72[b]	590.63±312.20[c]	406.49±261.75[a]	619.67±334.13[d]	803.40±211.24[e]	15.73	<0.001*

WBC=White blood cell; RBC=Red blood cell; HB=Haemoglobin; MEB=Monocytes, Eosinophils, Basophils; LYM = Lymphocytes; NEUT = Neutrophils; PLT = Platelet, HCT = Haematocrit. Values with different superscripts are significantly different ($p<0.05$)

In Table 4, comparison of haematological parameters among HIV-negative (control), HIV-infected, and HIV/HBV/HCV, HIV/HCV and HIV/HBV co-infected patients showed significant increase ($p<0.05$) in mean lymphocyte counts of 36.69±13.25, 42.02±12.75, 46.53±8.36, 47.64±14.35, and 49.61±5.81, and a significant decrease ($p<0.05$) in mean neutrophil counts of 54.43±13.52, 46.33±13.04, 44.23±9.30, 41.66±12.94 and 40.86±7.56 respectively. The mean platelet count (10^9/L) in HIV-negative control, HIV-infected, and HIV/HCV, HIV/HBV and HIV/HBV/HCV co-infected patients showed significant decrease ($p<0.05$) of 235.25±109.52, 229.26±104.70, 152.25±56.64, 138.69±56.25, and 130.33±79.51, as well as a significant decrease in CD4 cell counts (cells/µl) of 803.40±211.24, 619.67±334.13, 590.63±312, 550.15±311.72, and 406.49±261.75 respectively.

Discussion:

Although there have been drastic improvements in level of survival and HIV progression due to the use of combination antiretroviral therapy (ART), complications resulting from co-infections which could injure major organs of the body such as the liver, heart, kidney and others are now the major cause of death among HIV-infected patients. This study was conducted to compare the haematological parameters of HIV-patients co-infected by hepatitis B and C viruses with HIV-negative controls.

Our data showed that 40.0% of the recruited patients were HIV-infected on ART, 36.0% were HIV-infected but ART-naive while 24.0% were HIV-negative control, and male (40.2%) to female (59.8%) ratio was 1:1.5. The high ratio for females may be indicative of the fact that females tend to access hospital care more commonly than males, and not necessarily that more females are HIV-infected. The age groups 20-29 and 30-39 years were most commonly HIV-infected, with 26.7% and 23.7% of the total study population respectively. These age groups are sexually active and are usually involved in risky behaviors that could expose them to sexually transmitted infections such as HIV, HBV and HCV.

The mean values of some haematological parameters (total white blood count, red

blood cell, haemoglobin and haematocrit) were not significantly different between HIV-infected patients (on ART and ART-naive) compared to HIV-negative controls (*p*>0.05). However, there was significant increase in the mean values of MEB (monocytes, eosinophils and basophils) and lymphocytes, and a decrease in the mean values of neutrophils and platelets (*p*<0.05) between HIV-infected patients and HIV-negative controls. These findings agree with those of other studies (2,15) on the types of cytopenia commonly observed among HIV-infected individuals. The significant decrease in neutrophil and platelet counts in HIV co-infections indicate that co-infection suppresses the bone marrow and may increase the chances of cytopenia. This could be the result of increased oxidative stress caused by cytokines and reduction of oxidant status of co-infected patients. Our findings agree with others (16) showing that neutropenia and thrombocytopenia are common in viral infections such as HIV and hepatitis.

Although there was a significant increase (*p*<0.05) in the mean values of lymphocytes and monocytes in HIV-mono and co-infected patients in our study compared to HIV-negative controls, there was no significant difference (*p*>0.05) in the values of the red blood cells. It has been reported that both HIV and HBV/or HCV infections exert significant effect on the white blood cells rather than the red blood cells (15). In spite of the initiation of ART, alteration in haematological parameters can impact negatively on HIV-infected patients especially in those with co-infection of hepatitis B or C virus (10). The decrease in neutrophils and platelets among co-infected individuals could be attributed to synergistic effects of HIV and HBV/or HCV on the bone marrow with resultant effect of bone marrow failure, destruction of peripheral cells and invasion by opportunistic pathogens. Thrombocytopaenia in co-infected patients can lead to immune complex-mediated platelet clearance and anti-platelet HIV antibodies that can cross react with platelet membrane glycoprotein, creating a situation could lead to serious bleeding (17).

In this study, there was no significance difference in all the haematological parameters HIV-infected patients on ART with respect to gender (*p*>0.05). Although, the mean haemoglobin concentration in males (11.15±2.6 g/dl) was higher than the value in females (10.55±2.2 g/dl), this difference was not statistically significant (*p*=0.32). This contradicts previous findings of studies conducted in Nigeria and Uganda where significantly higher haemoglobin values were reported in HIV-infected females on ART than their than male counterpart (18,19).

The lower but insignificant mean haemoglobin concentration in HIV-infected females in our study could be associated with iron deficiency commonly experienced during the menstrual cycle especially as most of the participants in our study were within child-bearing age.

The mean CD4 cell count in males (729 cells/µl) was however significantly higher than in females (621 cells/µl) (*p*=0.007). It is expected that CD4 cell counts will drop as HIV infection progresses, and initiation of ART is meant to increase the CD4 cell count, but in the presence of other co-infections, CD4 cells are further reduced. In our study, there was statistically significant reduction in the mean CD4 cell count in HIV mono-infected (619 cells/µl) patients, and in HIV/HCV (590 cells/µl), HIV/HBV (550 cells/µl), and HIV/HBV/HCV (406 cells/µl) co-infected individuals, compared to HIV-negative controls (803 cells/µl). This finding is at variance with the mean CD4 count of 141.6 cells/µl and 121 cells/µl reported in studies conducted in South Africa and Nigeria respectively (provide reference!!). This disparity may be due to differences in the immune status of the individuals in the study or due to viral hepatitis. In individuals who have both HIV and HBV infections, there may be high HIV and HBV viral replication that may further contribute to impairment of the immune system of the patients.

The mean CD4 count (590 cells/µl) in HIV/HCV co-infected patients reported in our study was comparably higher than the mean CD4 count of 274 cells/µl, 260 cells/µl and 288.6 cells/µl reported from studies conducted in Nigeria, Ethiopia and India respectively (18,19). The reason for this disparity is unclear, however, this finding agrees with other study in Nigeria (20), indicating that those with HIV/HCV co-infection tend to present at earlier stages of the disease when immunity may still be strong.

Conclusion:

Our study showed that levels of white blood cells such as lymphocytes, neutrophils, platelets, and CD4 cells, which are markers of some haematological disorders like leukaemia and bleeding, were significantly affected by HIV co-infection with hepatitis B and C viruses. Screening for HBV and HCV should be routinely done for every HIV-infected patient, and these haematological parameters should be monitored regularly for better management of the patients.

Conflict of interest:

Authors declare no conflict of interest

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Original Article   **Open Access**

Bacteriological quality of community well water and public health concerns in Enugu urban, Nigeria

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Abstract:

Background: Water is a basic necessity used by humans for both domestic and industrial uses. Next to air, water is essential to life. It takes up about 71% of the earth's surface. The objective of this study is to determine the bacteriological quality of well water in Enugu urban, Nigeria

Methodology: A total of 60 domestic wells were selected from Abakpa, Obiagu and Achara layouts in Engu urban, Nigeria by stratified random sampling method, with 20 wells selected from each area based on location of well sites and construction parameters. Water samples were collected from each well using a sterile 200ml plastic bottle for bacteriological analysis to estimate total bacteria count in colony forming unit (cfu)/ml, total coliform count in most probable number (mpn)/100ml, and faecal coliform count in most probable number (mpn)/100ml. Bacterial isolates were identified using Gram reaction and conventional biochemical tests including catalase and coagulase for Gram positive bacteria, and oxidase, citrate utilization, hydrogen sulfide, indole, urease, methyl red, Voges Proskauer, and sugar fermentation tests for Gram negative bacteria. Antibiotic susceptibility testing (AST) of each isolate was performed by the disk diffusion method against selected antibiotics including penicillin G (10µg), ciprofloxacin (5µg), streptomycin (10µg), amoxicillin-clavulanic acid (20/10µg), and trimethoprim-sulfamethoxazole (25µg), and result interpreted using the European Committee for Antimicrobial Susceptibility Testing (EUCAST) break points. Comparative statistics of the data was performed using analysis of variance (ANOVA) with $p<0.05$ considered statistically significant.

Results: The well water in the three layouts were heavily contaminated as shown by comparatively high mean total bacteria counts of $0.8825\pm0.66\times10^4$ cfu/ml, $0.8435\pm0.6413\times10^4$ cfu/ml, and $0.8384\pm0.5948\times10^4$ cfu/ml for Abakpa, Obiagu and Achara layouts respectively ($p=0.9714$). The mean total coliform counts were 5.15 ± 5.284, 5.45 ± 4.31 and 5.05 ± 4.763 mpn/100ml ($p=0.8038$), and the mean faecal coliform counts were 2.4 ± 3.393, 2.65 ± 2.796 and 2.05 ± 2.35 mpn/100ml ($p=0.9631$) for Abakpa, Obiagu and Achara layouts respectively. A total of 50 pathogenic bacterial isolates were identified; *Klebsiella pneumoniae* 21 (43.8%), *Escherichia coli* 13 (30.0%), *Proteus* spp 6 (12.5%), *Pseudomonas aeruginosa* 6 (12.5%), and *Staphylococcus aureus* 2 (4.2%). The AST result shows that 75% of *K. pneumoniae, E. coli*, *Proteus* spp and *S. aureus* were resistant to all five antibiotics tested.

Conclusion: These findings showed high faecal contamination of domestic well water sources, which poses a significant infection risk to the community. Proper water treatment measures and personal hygiene practices are recommended, and well sites should be located at a safe distance from septic tanks, pit latrines, flowing gutters and refuse dump sites.

Keywords: domestic well water; quality; bacteria pathogens; antibiotic resistance; faecal contamination

Qualité bactériologique de l'eau de puits communautaire et problèmes de santé publique dans la ville d'Enugu, au Nigeria

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Résumé:

Contexte: L'eau est une nécessité fondamentale utilisée par les humains à la fois pour des usages domestiques et industriels. A côté de l'air, l'eau est essentielle à la vie. Il occupe environ 71% de la surface terrestre. L'objectif de cette étude est de déterminer la qualité bactériologique de l'eau de puits dans la ville d'Enugu, au Nigeria
Méthodologie: Un total de 60 puits domestiques ont été sélectionnés parmi les plans Abakpa, Obiagu et Achara dans la ville d'Enugu, au Nigeria, par une méthode d'échantillonnage aléatoire stratifié, avec 20 puits sélectionnés dans chaque zone en fonction de l'emplacement des sites de puits et des paramètres de construction. Des échantillons d'eau ont été prélevés dans chaque puits à l'aide d'une bouteille en plastique stérile de 200 ml pour une analyse bactériologique afin d'estimer le nombre total de bactéries dans l'unité formant colonie (cfu)/ml, le nombre total de coliformes dans le nombre le plus probable (mpn)/100 ml et le nombre de coliformes fécaux dans la plupart des cas. nombre probable (mpn)/100 ml. Les isolats bactériens ont été identifiés à l'aide de la réaction de Gram et de tests biochimiques conventionnels, notamment la catalase et la coagulase pour les bactéries Gram positives, et l'oxydase, l'utilisation du citrate, le sulfure d'hydrogène, l'indole, l'uréase, le rouge de méthyle, Voges Proskauer et les tests de fermentation des sucres pour les bactéries Gram négatives. Le test de sensibilité aux antibiotiques (AST) de chaque isolat a été effectué par la méthode de diffusion sur disque contre des antibiotiques sélectionnés, notamment la pénicilline G (10 µg), la ciprofloxacine (5 µg), la streptomycine (10 µg), l'amoxicilline-acide clavulanique (20/10 µg) et le triméthoprime-sulfaméthoxazole. (25 µg) et résultat interprété à l'aide des points de rupture du Comité européen pour les tests de sensibilité aux antimicrobiens (EUCAST). Les statistiques comparatives des données ont été réalisées à l'aide d'une analyse de variance (ANOVA) avec $p<0,05$ considéré comme statistiquement significatif.
Résultats: L'eau du puits dans les trois aménagements était fortement contaminée, comme le montrent les taux de bactéries totaux moyens relativement élevés de $0,8825\pm0,66\times10^4$ UFC/ml, $0,8435\pm0,6413\times10^4$ UFC/ml et $0,8384\pm0,5948\times10^4$ UFC/ml pour Dispositions Abakpa, Obiagu et Achara respectivement ($p=0,9714$). Le nombre moyen de coliformes totaux était de $5,15\pm5,284$, $5,45\pm4,31$ et $5,05\pm4,763$ mpn/100 ml ($p=0,8038$), et le nombre moyen de coliformes fécaux était de $2,4\pm3,393$, $2,65\pm2,796$ et $2,05\pm2,35$ mpn/100 ml ($p=0.9631$) pour les dispositions Abakpa, Obiagu et Achara respectivement. Un total de 50 isolats bactériens pathogènes ont été identifiés; *Klebsiella pneumoniae* 21 (43,8%), *Escherichia coli* 13 (30,0%), *Proteus* spp 6 (12,5%), *Pseudomonas aeruginosa* 6 (12,5%) et *Staphylococcus aureus* 2 (4,2%). Le résultat de l'AST montre que 75% des *K. pneumoniae, E. coli, Proteus* spp et *S. aureus* étaient résistants aux cinq antibiotiques testés.
Conclusion: Ces résultats ont montré une contamination fécale élevée des sources d'eau de puits domestiques, ce qui pose un risque d'infection important pour la communauté. Des mesures appropriées de traitement de l'eau et des pratiques d'hygiène personnelle sont recommandées, et les sites de puits doivent être situés à une distance de sécurité des fosses septiques, des latrines à fosse, des gouttières et des décharges.

Mots-clés: eau de puits domestique; qualité; bactéries pathogènes; résistance aux antibiotiques; contamination fécale

Introduction:

A well is a type of ground water facility and a major source of water. Modern and treated drilled wells are commonly found in developed countries but in developing countries like Nigeria, with faulty sanitation, access to treated well water is uncommon. This has weakened the health, education and economic activities of the citizens (1,2). In past times, ground water, mostly deep, confined aquifers have been considered to be least susceptible to microbial contamination of human origin. However, protected deep wells can be contaminated where there is a hydrologic connection between these wells and a faulty septic tank or sewer line pit latrines, or contaminated lagoons or rivers. This situation is common in rural and semi-urban environments (3,4).

Access to adequate safe drinking water is an important necessity for every community but many developing regions of the world still lack a steady supply of potable water. Poverty, illiteracy and inadequate sanitary hygiene have also led to an increase in water borne diseases and environmental pollution (5,6). In developing countries, children are the most vulnerable to water borne diseases causing more than 20 million deaths (7). It is speculated that accessible potable water will increase to by only ten per cent in the next thirty years while the earth's population is projected to rise by one third. Except there is an efficient rise in water use, this imbalance will reduce quality water services, diminish the health conditions of the people and deteriorate the environment and globe (8).

Ground water is an age-old alternative source of water used in most households in

Bacteriological quality of well water

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Nigeria for domestic purposes. As such, it is a common feature in most residences [9]. A major challenge is the high microbial contaminants found in such wells due to the proximity to contents of septic tanks, open drainages, leachates of dumpsites, flood, soil matter, and agricultural wastes. Inadequate routine disinfection of these wells predisposes an average of 6 to 20 persons (depending on the number of residents) to water borne diseases caused by a variety of pathogenic microbes including enteric bacteria. These microorganisms include *Escherichia coli, Klebsiella* spp*, Shigella, Salmonella, Enterobacter, Citrobacter* spp*, Campylobacter jejuni, Edwardsiella, Hafnia, Serratia, Yersinia, Morganella, Erwinia*, *Providencia* spp, *Pseudomonas* spp*, Proteus* spp*, Acinetobacter* spp*,* and a plethora of other unidentified or unidentifiable bacteria that are transmitted through direct or indirect contamination of water sources by human faeces and waste water (10,11). Some of the diseases caused include dysentery, typhoid fever, paratyphoid fever, infantile paralysis, and hepatitis. These diseases affect about 1.7 billion people worldwide, leading to some 2.2 million deaths annually (12).

The microbiological quality if drinking water is assessed by testing for non-pathogenic bacteria of faecal origin. Microorganisms used as indicators of water quality are coliforms, faecal *Streptococci*, *Clostridium perfringes* and *Pseudomonas aeruginosa* (13). Oguntoke et al., (14) reported that poor well construction, and proximity to point source of contamination make wells vulnerable to microbial pollution. Higher populations of *E. coli* and *Klebsiella* have been found in hand-dug wells during the wet season than during the dry season due to faeces-contaminated flood waters which seep through cracks into the wells (10,15). In line the above reported findings, it was important to determine the microbial quality of domestic wells in three major densely populated areas in Enugu town.

Materials and method:

Study setting and sampling

Randomly selected areas for this study were Abakpa, Obiagu, and Achara layouts, all located in Enugu urban. Enugu is a State in south-east geopolitical zone of Nigeria. It is the 29th largest in area and 22nd most populous, with an estimated population of over 4.4 million as at 2016. The latitude of Enugu, is 6.459964, and the longitude is 7.548949, with GPS coordinates of 6° 27' 35.8704'' N and 7° 32' 56.2164'' E. It is a center of mining, education and commerce. Majority of individuals who reside in these areas are either middle-income or low-income earners both in the public and private sectors of the economy. Well water constitutes the major source of water for domestic purposes in the areas.

Following interactions with the inhabitants, we discovered that wells were constructed with concrete and on the average, are 8 metres deep. Wells for the study were randomly selected, with 20 wells in each layout based on their location (close to septic tank, flooded drainage, dump sites and outdoor sites), and based on wall construction types (good casing, cracked casing, ground level, and ground level with cracked casing).

Collection of water samples

Water samples were collected following the method described by Idowu et al., (16). Water sample from each well was aseptically collected into a sterile 200 ml plastic bottle tied with a strong string to a piece of metal of about 500g. The bottle cap was removed and bottle immersed into the well to a depth of 2 metres. The bottle was then brought up to the surface without touching the sides of the well and was immediately covered. The bottles were placed in cool boxes and transported to the laboratory within 4 hours for analysis.

Total bacteria count

Each of the water samples was serially diluted to a 6-fold dilution. Approximately 9 ml of distilled water was dispensed into 6 labeled tubes. Using a sterile pipette, 1 ml of each water sample was transferred into the first tube. Subsequently, 1 ml was serially diluted into the other tubes. From the 10^{-2} dilutions, 1 ml of water sample was dropped into a sterile Petri dish, and a well-prepared sterile nutrient broth was poured into the Petri dish using pour plate technique. This was properly mixed, allowed to set and incubated at 37°C for 24 hours. The colonies formed were counted and expressed as cfu/ml.

Total coliform and faecal coliform count

The multiple tube fermentation technique described by Collins and Lynne and by American Public Health Association was used (17,18) to determine total and faecal coliform counts. In this method, single and double strength MacConkey broth were prepared in graduated glass flasks, sterilized by autoclaving at 121°C for 15 minutes and dispensed into sterile test tubes containing inverted sterile Durham's tubes. For the presumptive test, from each water sample, 50 ml of water sample was inoculated into a 50 ml double strength MacConkey broth, 10 ml of water sample was inoculated into each of the five 10 ml double strength broth and

1 ml of water sample into each of the five 1 ml single strength broth. The tubes were incubated at 37°C for 24 hours and observed for acid and gas production. Sterile distilled water was used as a control for each test batch.

Presumptive coliform count was determined by the most probable number (MPN) of coliform per 100 ml of water sample using the McCrady's probability tables as reference after combining the various positive and negative results (19). For confirmatory coliform count, a loopful of broth from each positive tube was sub-cultured into a fresh tube of MacConkey broth and incubated at 44°C for 24 hours. Gas production was observed in positive tubes.

Identification of bacterial isolates

Positive tubes from presumptive and confirmatory coliform tests were sub-cultured on Eosin Methylene Blue (EMB) agar for enumeration of faecal coliforms and on nutrient agar. All the inoculated media were incubated at 37°C for 24 hours. Pure isolates were characterized using Gram stain microscopy and conventional biochemical tests as described by Agwaranze et al., (20). The biochemical tests used to characterize Gram-positive isolates were catalase and coagulase tests, while for the Gram-negative isolates, biochemical tests used include oxidase, urease, indole, methyl red, Voges-Proskauer (VP), citrate utilization, h**y**drogen sulfide, and sugar fermentation tests (21).

Antibiotic susceptibility testing

Isolated bacteria were tested against five selected antibiotics; penicillin G (10μg), ciprofloxacin (5μg), streptomycin (10μg), amoxicillin-clavulanic acid (20/10μg), and sulfamethoxazole-trimethoprim (25μg). AST was performed using modified Kirby-Bauer disk diffusion method (22). Briefly, pure colonies of each test bacterium that have been cultured on nutrient agar overnight were used to prepare inoculum in nutrient broth, which was standardized by comparing with 0.5 MacFarland turbidity standards. The inoculum was then streaked on Mueller-Hinton agar plate with a sterile cotton swab. The plate was allowed to dry for 5 min and antibiotics disks were placed on the surface of the agar using sterile forceps. The plates were inverted and incubated aerobically for 24 hours at 37°C. A calibrated ruler was used to measure the diameter of the zone of inhibition around each antibiotic disk. Sensitivity or resistance of each isolate to the antibiotics was determined using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (23). *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used as control strains.

Statistical analysis

Data were presented as mean±SD and analysis was done using GraphPad software (San Diego, USA). Comparison of mean values of variables was done with one way analysis of variance (ANOVA), and *p* value less than 0.05 was considered statistically significant.

Results:

The bacteriological analysis of domestic well water samples shows that in Abakpa, the total bacteria count ranged from $0.05\text{-}2.22\times10^4$ cfu/ml (mean of $0.8825\pm0.6604 \times 10^4$ cfu/ml), total coliform count from 0-17 mpn/100ml (mean of 5.15±5.284 mpn/10m) and faecal coliform count of 0-11 mpn/100ml (mean of 2.4± 3.393 mpn/100ml) (Table 1a & 1b). In Obiagu, the mean total bacteria count ranged from $0.06\text{-}2.58\times10^4$ cfu/ml (mean of $0.8435\pm0.6413\times10^4$ cfu/ml), total coliform count of 0-13 mpn/100ml (mean of 5.45±4.31 mpn/100ml), and faecal coliform count of 0-9 mpn/100ml (mean of 2.65 ± 2.796 mpn/100ml) (Table 2a & 2b). In Achara layout, the total bacteria count ranged from $0.13\text{-}1.86\times10^4$ cfu/ml (mean of $0.8385\pm0.5948 \times10^4$ cfu/ml), total coliform count of 0-17 mpn/ 100ml (mean of 5.05±4.763 mpn/100ml), and faecal coliform count of 0-7 mpn/100ml (mean of 2.05±2.35 mpn/100ml) (Table 3a & 3b).

Statistical analysis by ANOVA shows that the total bacteria counts were not significantly different between the three layouts (*p*= 0.9714). Similarly, the total coliform count was not significantly different between the three layouts (*p*=0.9631) and likewise for the faecal coliform count (*p*=0.8038).

With respect to the site location of the wells, wells located close to septic tanks (S) had the highest total bacterial and coliform counts followed by wells located close to flooded drainage (F) and dumpsites (DS) while the wells at outdoor (O) sites had the lowest total bacterial counts and did not contain coliforms (Tables 1a, 2a & 3a). With regard to the construction types, wells with cracked casing (CC) had the highest total bacterial and coliform counts followed by wells at ground level (GL) and wells at ground level with cracked casing (GL/CC), while wells with good casing (GC) had the least total bacterial count and had no coliforms (Tables 1b, 2b & 3b).

A total of 50 bacterial isolates were cultured from water samples in the three layouts (Table 4). The isolates were *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus* spp and *Staphylococcus aureus*. *Klebsiella pneumoniae* (43.8%) was the most frequent bacterial isolated while *S. aureus* (4.2%)

was the least frequent bacteria. The bacterial isolates from the wells in the three layouts showed multiple resistance to the antibiotics tested (Tables 5, 6 & 7), except *P. aeruginosa* in Well 4 (S1) in Obiagu (Table 6) and *K. pneumoniae* in Well 2 (F1) in Achara (Table 7), which showed intermediate resistance to sulphamethoxazole-trimethoprim (SXT).

Table 1a: Bacteriological analysis of domestic well water samples in Abakpa from different well sites

Site of well water samples	Total bacteria count (10⁴CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Bacteria isolated from samples
S1	2.22	17	0	*Proteus* spp
S2	1.94	13	11	*K. pneumoniae*
				E. coli
S3	1.32	11	8	*E. coli*
S4	0.83	6	4	*E. coli*
S5	0.81	4	4	*E. coli*
F1	1.24	8	0	*Proteus* spp
F2	1.19	9	6	*K. pneumoniae*
				E. coli
F3	2.20	12	7	*K. pneumoniae*
F4	1.06	0	0	*S. aureus*
F5	0.63	9	0	*P. aeruginosa*
DS1	0.95	2	0	*P. aeruginosa*
DS2	0.89	6	4	*K. pneumoniae*
DS3	0.14	0	0	
DS4	0.77	4	4	*K. pneumoniae*
DS5	0.50	2	0	*P. aeruginosa*
O1	0.26	0	0	
O2	0.18	0	0	
O3	0.24	0	0	
O4	0.23	0	0	
O5	0.05	0	0	
Mean count	0.8825	5.15	2.4	
SD	0.6604	5.284	3.393	
Range	0.05-2.22	0-17	0-11	

S = septic tank; F = flooded drainage; DS = dump site; O = outdoor site; SD = Standard deviation

Table 1b: Bacteriological analysis of domestic well water samples in Abakpa from different well construction types

Samples from different well construction types	Total bacteria count (10⁴CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Bacteria isolated from samples
0GC1	0.26	0	0	
GC2	0.18	0	0	
GC3	0.24	0	0	
GC4	0.14	0	0	
GC5	0.05	0	0	
CC1	2.22	17	0	*Proteus* spp
CC2	1.19	9	6	*K. pneumoniae*
				E. coli
CC3	1.32	11	8	*E. coli*
CC4	2.20	12	7	*K. pneumoniae*
				E. coli
CC5	0.23	0	0	
GL1	1.24	8	0	*Proteus* spp.
GL2	1.94	13	11	*K. pneumoniae*
				E. coli
GL3	1.06	0	0	*S. aureus*
GL4	0.50	2	0	*P. aeruginosa*
GL5	0.81	4	4	*E. coli*
GL/CC1	0.95	2	0	*P. aeruginosa*
GL/CC2	0.83	6	4	*E. coli*
GL/CC3	0.89	6	4	*K. pneumoniae*
GL/CC4	0.63	9	0	*P. aeruginosa*
GL/CC5	0.77	4	4	*K. pneumoniae*
Mean count	0.8825	5.15	2.4	
SD	0.6614	5.284	3.393	
Range	0.05-2.22	0-17	0-11	

GC = good casing; CC = cracked casing, GL = ground level; and GL/CC = ground level with cracked casing; SD=Standard deviation

Table 2a: Bacteriological analysis of domestic well water samples in Obiagu from different well sites

Sites of well water samples	Total bacteria count (10⁴CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Organisms isolated from samples
S1	1.01	13	6	*E. coli* *P. aeruginosa*
S2	2.58	11	9	*K. pneumoniae*
S3	0.98	9	6	*K. pneumoniae* *E. coli*
S4	1.89	13	8	*K. pneumoniae*
S5	0.69	7	4	*E. coli*
F1	1.15	6	2	*K. pneumoniae* *S. aureus*
F2	0.62	4	2	*K. pneumoniae*
F3	0.70	4	2	*K. pneumoniae*
F4	0.78	4	2	*K. pneumoniae*
F5	0.83	6	2	*K. pneumoniae*
DS1	0.73	4	0	*Proteus* spp.
DS2	0.92	7	4	*E. coli*
DS3	0.86	6	4	*K. pneumoniae*
DS4	1.76	11	0	*Proteus* spp
DS5	0.75	4	2	*K. pneumoniae*
O1	0.17	0	0	
O2	0.12	0	0	
O3	0.11	0	0	
O4	0.06	0	0	
O5	0.16	0	0	
Mean count	0.8435	5.45	2.65	
SD	0.6413	4.310	2.796	
Range	0.06-2.58	0-13	0-9	

S = septic tank; F = flooded drainage; DS = dump site; O = outdoor site; SD=Standard deviation

Table 2b: Bacteriological analysis of domestic well water samples in Obiagu from different well construction types

Samples from different well construction types	Total bacteria count (10⁴CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Organisms isolated from samples
GC1	0.17	0	0	
GC2	0.12	0	0	
GC3	0.11	0	0	
GC4	0.06	0	0	
GC5	0.16	0	0	
CC1	1.15	6	2	*K. pneumoniae* *S. aureus*
CC2	1.01	13	6	*E. coli* *P. aeruginosa*
CC3	0.98	9	6	*K. pneumoniae*
CC4	2.58	11	9	*K. pneumoniae* *Proteus* spp
CC5	0.69	7	4	*E. coli*
GL1	0.92	7	4	*E. coli*
GL2	0.86	6	4	*K. pneumoniae*
GL3	0.62	4	2	*K. pneumoniae*
GL4	0.78	4	2	*K. pneumoniae*
GL5	0.75	4	2	*K. pneumoniae*
GL/CC1	0.73	4	0	*Proteus* spp
GL/CC2	1.76	11	0	*Proteus* spp *P. aeruginosa*
GL/CC3	0.70	4	2	*K. pneumoniae*
GL/CC4	1.89	13	8	*K. pneumoniae*
GL/CC5	0.83	6	2	*K. pneumoniae*
Mean count	0.8435	5.45	2.65	
SD	0.6413	4.310	2.796	
Range	0.06-2.58	0-13	0-9	

GC = good casing; CC = cracked casing, GL = ground level; and GL/CC = ground level with cracked casing; SD = Standard deviation

Table 3a: Bacteriological analysis of domestic well water samples in Achara layout from different well sites

Site of well water samples	Total bacteria count (10⁴CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Organisms isolated from samples
S1	1.28	11	6	*E. coli*
S2	1.47	17	7	*E. coli*
S3	1.86	6	2	*K. pneumoniae*
S4	0.50	4	2	*E. coli*
S5	0.60	7	4	*E. coli*
F1	1.59	6	4	*K. pneumoniae*
F2	0.76	6	2	*K. pneumoniae*
F3	0.53	4	2	*E. coli*
F4	1.73	12	6	*K. pneumoniae* *E. coli*
F5	0.42	4	0	*Proteus* spp
DS1	1.72	7	0	*P. aeruginosa*
DS2	1.24	2	2	*K. pneumoniae*
DS3	0.79	2	0	*Proteus* spp
DS4	0.89	11	4	*K. pneumoniae*
DS5	0.55	2	0	*P. aeruginosa*
O1	0.18	0	0	
O2	0.21	0	0	
O3	0.15	0	0	
O4	0.17	0	0	
O5	0.13	0	0	
Mean count	0.8385	5.05	2.05	
SD	0.5948	4.763	2.350	
Range	0.13-1.86	0-17	0-7	

S = septic tank; F = flooded drainage; DS = dump site; O = outdoor site; SD = Standard deviation

Table 3b: Bacteriological analysis of domestic well water samples in Achara from different well construction types

Samples from different well construction types	Total bacteria count (CFU/ml×10⁴)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Organisms isolated from samples
GC1	0.18	0	0	
GC2	0.21	0	0	
GC3	0.15	0	0	
GC4	0.17	0	0	
GC5	0.13	0	0	
CC1	1.24	2	2	*K. pneumoniae*
CC2	1.28	11	6	*E. coli*
CC3	1.47	17	7	*E. coli*
CC4	0.50	4	2	*E. coli*
CC5	0.60	7	4	*E. coli*
GL1	1.72	7	0	*P. aeruginosa*
GL2	1.59	6	4	*K. pneumoniae*
GL3	0.76	6	2	*K. pneumoniae*
GL4	0.53	4	2	*E. coli*
GL5	0.42	4	0	*Proteus* spp
GL/CC1	0.79	2	0	*Proteus* spp
GL/CC2	0.89	11	4	*K. pneumoniae*
GL/CC3	1.86	6	2	*K. pneumoniae*
GL/CC4	1.73	12	6	*K. pneumoniae* *E. coli*
GL/CC5	0.55	2	0	*P. aeruginosa*
Mean count	0.8385	5.05	2.05	
SD	0.5948	4.763	2.350	
Range	0.13-1.86	0-17	0-7	

GC = good casing; CC = cracked casing, GL = ground level; and GL/CC = ground level with cracked casing; SD=Standard deviation

Table 4: Frequency distribution of bacteria isolates in the domestic well water samples from Abakpa, Obiagu and Achara Layouts, Enugu, Nigeria

Bacteria isolates	Frequency (%)
Klebsiella pneumoniae	21 (43.8)
Escherichia coli	15 (30.0)
Pseudomonas aeruginosa	6 (12.5)
Proteus spp	6 (12.5)
Staphylococcus aureus	2 (4.2)
Total	**50 (100)**

Table 5: Antibiotic sensitivity test of isolates from well water samples in Abakpa

Sample	Isolate	Zone Diameter (mm)				
		CPX	SXT	S	PN	AU
S1	*Proteus* spp	8	10	4	6	7
F1	*Proteus* spp	7	9	4	5	9
F2	*K. pneumoniae, E. coli*	8	7	2	3	8
DS1	*P. aeruginosa*	8	5	2	3	2
S2	*K. pneumoniae, E. coli*	9	3	3	5	10
S3	*E. coli*	7	6	4	6	8
F3	*K. pneumoniae*	6	4	2	5	10
S4	*E. coli*	5	6	5	3	7
DS2	*K. pneumoniae*	7	5	3	2	7
F4	*S. aureus*	6	5	2	2	5
F5	*P. aeruginosa*	9	13	5	2	4
DS4	*K. pneumonia*	7	5	3	5	9
DS5	*P. aeruginosa*	6	3	5	2	7
S5	*E. coli*	7	3	4	5	6

For *Enterobacteriaceae*: CPX=Ciprofloxacin (5µg): S ≥ 25mm, R < 22mm; SXT=Trimethoprim-sulfamethoxazole (25µg): S ≥ 14mm, R<11mm; S=Streptomycin (10µg): S ≥ -, R < -; PN=Penicillin (10µg): S≥14mm, R< 14mm); AU=Amoxicillin-clavulanic acid (20/10µg): S ≥ 19mm, R<19mm
For *Pseudomonas* spp: CPX: S ≥ 26mm, R<26mm; SXT: S ≥ -, R< -; S: S ≥ 16mm, R< 16mm; PN: S ≥ 18mm, R< 18mm; AU: S ≥ 16mm, R<16mm;
For *Staphylococcus* spp: CPX: S ≥ 21mm, R<21mm; SXT: S ≥ 17mm, R< 14mm; S: S ≥ 18mm, R< 18mm; PN: S ≥ 26mm, R<26mm; AU: S ≥ -, R< -
Antibiotics without breakpoint values have not been determined by EUCAST (2019). The solates were resistant to all the antibiotics by EUCAST guideline

Table 6: Antibiotic sensitivity test of isolates from well water samples in Obiagu

Sample	Isolate	Zone Diameter (mm)				
		CPX	SXT	S	PN	AU
DS1	*Proteus* spp	6	7	4	2	6
DS2	*E. coli*	7	8	3	4	5
F1	*K. pneumoniae, S. aureus*	10	6	6	5	8
S1	*E. coli, P. aeruginosa*	9	13	5	2	4
DS3	*K. pneumoniae*	12	6	7	2	8
DS4	*Proteus* spp	6	6	5	3	8
F2	*K. pneumoniae*	8	4	3	2	9
F3	*K. pneumoniae*	8	6	4	3	6
S3	*K. pneumoniae, E. coli*	7	3	4	4	7
S4	*K. pneumoniae*	14	9	2	5	10
F5	*K. pneumoniae*	7	6	2	1	7
F4	*K. pneumoniae*	7	7	2	3	6
S2	*K. pneumoniae*	13	8	5	2	8
DS5	*K. pneumoniae*	15	7	3	3	7
S5	*E. coli*	7	7	3	3	7

For *Enterobacteriaceae*: CPX=Ciprofloxacin (5µg): S ≥ 25mm, R < 22mm; SXT=Trimethoprim-sulfamethoxazole (25µg): S ≥ 14mm, R<11mm; S=Streptomycin (10µg): S ≥ -, R < -; PN=Penicillin (10µg): S≥14mm, R< 14mm); AU=Amoxicillin-clavulanic acid (20/10µg): S ≥ 19mm, R<19mm
For *Pseudomonas* spp: CPX: S ≥ 26mm, R<26mm; SXT: S ≥ -, R< -; S: S ≥ 16mm, R< 16mm; PN: S ≥ 18mm, R< 18mm; AU: S ≥ 16mm, R<16mm;
For *Staphylococcus* spp: CPX: S ≥ 21mm, R<21mm; SXT: S ≥ 17mm, R< 14mm; S: S ≥ 18mm, R< 18mm; PN: S ≥ 26mm, R<26mm; AU: S ≥ -, R< -
Antibiotics without breakpoint values have not been determined by EUCAST (2019). The bacterial isolates were all resistant to the antibiotics except *P. aeruginosa* (Well 4, S1) which showed intermediate resistance to SXT

Table 7: Antibiotic sensitivity test of isolates from well water samples in Achara layout, Enugu, Nigeria

Sample	Isolate	Zone Diameter (mm)				
		CPX	SXT	S	PN	AU
DS1	*P. aeruginosa*	5	5	7	5	6
F1	*K. pneumoniae*	15	11	5	2	8
DS2	*K. pneumoniae*	9	5	7	3	8
F2	*K. pneumoniae*	8	6	5	3	6
DS3	*Proteus* spp	6	5	7	4	6
DS4	*K. pneumonia*	8	8	7	2	7
S1	*E. coli*	5	4	6	7	6
S2	*E. coli*	12	8	4	8	6
S3	*K. pneumoniae*	8	8	5	4	7
S4	*E. coli*	5	7	4	6	7
F3	*E. coli*	9	7	3	6	6
F4	*K. pneumoniae*	10	5	8	4	5
S5	*E. coli*	6	5	6	6	7
DS5	*P. aeruginosa*	7	5	3	5	4
F5	*Proteus* spp	9	8	4	5	8

For *Enterobacteriaceae*: CPX=Ciprofloxacin (5µg): S ≥ 25mm, R < 22mm; SXT=Trimethoprim-sulfamethoxazole (25µg): S ≥ 14mm, R<11mm; S=Streptomycin (10µg): S ≥ -, R < -; PN=Penicillin (10µg): S≥14mm, R< 14mm); AU=Amoxicillin-clavulanic acid (20/10µg): S ≥ 19mm, R<19mm
For *Pseudomonas* spp: CPX: S ≥ 26mm, R<26mm; SXT: S ≥ -, R< -; S: S ≥ 16mm, R< 16mm; PN: S ≥ 18mm, R< 18mm; AU: S ≥ 16mm, R<16mm;
For *Staphylococcus* spp: CPX: S ≥ 21mm, R<21mm; SXT: S ≥ 17mm, R< 14mm; S: S ≥ 18mm, R< 18mm; PN: S ≥ 26mm, R<26mm; AU: S ≥ -, R< -
(EUCAST, 2019). The bacterial isolates were all resistant to the antibiotics except isolates in Well 2 (F1) which showed intermediate resistance to SXT.

Discussion:

The presence of microorganisms determines the sanitary quality of potable water. The guidelines set by the World Health Organization (WHO) approve coliforms, especially *E. coli* as standard indicator organisms. These organisms mainly of faecal origin are pathogenic to man. Ideally, water for drinking, cooking or cleaning purposes should not contain organisms of faecal origin (24). The presence of these bacteria in water is a likely indication of the presence of pathogenic microbes such as bacteria, viruses and parasites. Furthermore, detection of these organisms, especially *E. coli*, in water samples confirms recent faecal contamination, due to the fact that the organism does not survive for long period outside its normal host, which is the gastrointestinal tract of animals (7). The presence of these faecal organisms could be attributed to contamination of the wells by sewage, flooded drainages, poor construction, and poor sanitation.

The findings of high total bacteria count in well water samples from Abakpa (0.05-22.2×10⁴ cfu/ml), Obiagu (0.06-2.58×10⁴ cfu/ml) and Achara (0.13-1.86×10⁴cfu/ml) layouts in our study is similar to those of Onuorah et al., (10) and Agwaranze et al., (20), who reported high coliform counts, indicating that these wells were highly contaminated with bacterial pathogens that can serve as possible cause of water borne diseases. The high bacterial counts may be attributed to run-off water and flooded drainages, which could have possibly entered into some wells during the rainy season and dirt particles from the environment. The total coli-

form count ranged from 0-17 mpn/100ml in samples from wells in Abakpa, 0-13 mpn /100ml from Obiagu, and 0-17 mpn/100ml from Achara layout. These values exceed the recommended limit of 0 mpn/100ml coliforms in water by the World Health Organization (24).

In the three areas under study, wells located close to septic tanks (S) were grossly contaminated with coliforms while wells located close to dumpsites (DS) and flooded drainages (F) were mildly contaminated, but all the wells at outdoor (O) sites did not contain coliforms. These findings agree with the study conducted in Sagamu by Idowu et al., (16), who reported high numbers of pathogenic organisms of faecal origin from poorly constructed wells. Agwaranze et al., (20) also reported that well water used as source of water for domestic purposes in Wukari was grossly contaminated with faecal coliform bacteria. The siting of the wells close to septic tanks and percolation of sewage inside the wells were possible reasons for contamination by faecal coliforms. Studies in Kenya showed decreased coliform contamination of wells that are situated far from septic tanks (7).

In our study, wells with cracked casing (CC) had the highest bacterial count and coliform contamination, those at ground level (GL) and ground level with cracked casing (GL/CC) also had coliform contamination, but wells with good casing (GC) had no coliform contaminations. These results agreed with the findings of similar studies conducted in Awka (10) and Kaduna (25) in Nigeria where high contaminations of wells by coliforms were reported. The high number of coliforms may be due to the percolation and entry of sewage into ground

water through cracked casings (15), poor construction of the wells and poor environmental conditions of well locations (13). In Ibadan, wells with cracked casings showed high possibilities of contamination from pollutants seeping into them. Furthermore, wells located at ground level were prone to high contamination from materials that gained entrance from the surface.

The frequency distribution of the 50 bacteria isolates is similar to the findings of Agwaranze et al.*,* (20) who isolated *E. coli*, *K. pneumoniae*, *Salmonella typhi, P. aeruginosa, P. vulgaris*, and *S. aureus* from wells in Wukari, Taraba State, Nigeria. Idowu et al., (16) in Sagamu, Ogun State, Nigeria also reported wells that were highly contaminated with faecal coliforms including *E. coli, Klebsiella* spp*, Salmonella* spp, and *Pseudomonas* spp. In microbiological quality assessment of ground water in West Thrace, Turkey, Aydin (26) reported total coliforms, thermo-tolerant coliforms, *E. coli, Salmonella* spp and *P. aeruginosa* isolated in 25%, 17.5%, 15%, 15% and 15% of the ground water samples respectively. Ibiebele and Sokari (27) isolated *E. coli, Enterococcus faecalis, Pseudomonas* spp, *Klebsiella* spp, *Staphylococcus* spp., *Proteus* spp, *Aeromonas* spp, *Chromobacterium* spp, *Flavobacterium* spp, and *Serratia* spp from wells in shanty settlements in Port Harcourt, Nigeria.

The antibiotic susceptibility testing on our isolates showing multiple resistance to antibiotics, is similar to the study of Mishra et al., (28) who reported coliforms from water samples that were resistant to multiple antibiotics. Although bacterial contaminations of the wells in the three layouts were comparable (*p*=0.9714), wells in Achara and Obiagu had the lower contamination rates than wells in Abakpa. The presence of these pathogenic bacteria in some of the well water samples studied could constitute potential public health hazard to the consumers. High morbidity from enteric diseases such as diarrhoea, dysentery and typhoid fever in Nigeria may be due to widespread consumption of contaminated well water. Therefore, such wells must be adequately treated to protect the health of the consumers.

Conclusion:

Our study showed that domestic wells in Abakpa, Obiagu, and Achara layouts of Enugu urban in Nigeria were highly contaminated by bacteria above the recommended safety levels for domestic water consumption, and presence of coliform bacteria in the water samples indicated recent faecal pollution. Wells located close to septic tanks, dumpsites and flooded drainage,

and those with cracked casing and at ground level were more highly contaminated by coliform bacteria. Therefore, to avert the spread of water borne diseases, it is necessary to treat well water before domestic use. Residents of the areas need to be educated on proper hygienic practices and waste disposal. The recommended minimum distance of 15 feet apart between septic tanks, pit latrines, flowing gutters, refuse dump sites and wells should also be observed during well construction.

Conflict of interest:

Authors declare no conflict of interest.

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Original article   **Open Access**

Molecular detection of vector-borne bacterial pathogens in dromedary camels from Algeria

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Abstract:

Background: In Algeria, little focus was placed on camels as hosts of tick-borne bacterial diseases. Recent studies showed a high prevalence of tick infestation in dromedaries. Transmission of various pathogenic microorganisms to camels by ticks imposes considerable economic losses to livestock and greatly impact on human and animal health. The aim of our study was to investigate the occurrence of vector-borne zoonotic bacteria in camels from Algeria.
Methodology: Blood samples were collected from 80 randomly selected camels in Laghouat province, southern Algeria. The samples were screened for *Anaplasma* spp, *Bartonella* spp, *Rickettsia* spp and *Coxiella burnetii* by qPCR. All positive samples were confirmed by standard PCR followed by sequencing. Data on age, sex, tick infestation and location of the camels were analyzed using the SPSS version 17.0 and association of these with vector-borne bacterial pathogens was determined using Chi-square (χ^2) test. P value lower than 0.05 was considered as indicative of significance.
Results: Twenty five of the 80 (31.3%) camels were positive to at least one vector-borne bacterial pathogen with *Anaplasma phagocytophilum* (22.5%, 18/80) being the most prevalent species, followed by *Anaplasma platys* (7.5%, 6/80) and *Bartonella dromedarii* (2.5%, 2/80). Only one camel was co-infected with two pathogens. All samples tested negative for *Rickettsia* spp and *Coxiella burnetii*. None of the factors (age, sex, tick infestation and study sites) was significantly associated with prevalence of vector-borne bacteria in the camels (*p*>0.05).
Conclusion: The present study is the first report of anaplasmosis and bartonellosis in *"Camelus dromedaries"* from Algeria. Our results highlighted the need for further investigations on tickborne pathogens of camels.

Keywords: *Anaplasma phagocytophilum*, *Anaplasma platys, Bartonella*, tick borne bacteria, molecular detection, dromedary camels, Algeria

Détection moléculaire des pathogènes bactériens à transmission vectorielle chez les dromadaires d'Algérie

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Résumé

Contexte: En Algérie, peu d'attention a été accordée aux chameaux en tant qu'hôtes de maladies bactériennes transmises par les tiques. Des études récentes ont montré une forte prévalence d'infestation par les tiques chez les dromadaires. La transmission de divers micro-organismes pathogènes aux chameaux par les tiques impose des pertes économiques considérables au bétail et a un impact considérable sur la santé humaine et animale. Le but de notre étude était d'étudier la présence de bactéries zoonotiques à transmission vectorielle chez les chameaux d'Algérie.

Méthodologie: Des échantillons de sang ont été prélevés sur 80 chameaux sélectionnés au hasard dans la province de Laghouat, dans le sud de l'Algérie. Les échantillons ont été criblés pour *Anaplasma* spp, *Bartonella* spp, *Rickettsia* spp et *Coxiella burnetii* par qPCR. Tous les échantillons positifs ont été confirmés par PCR standard suivie d'un séquençage. Les données sur l'âge, le sexe, l'infestation par les tiques et l'emplacement des chameaux ont été analysées à l'aide de la version SPSS 17.0 et l'association de celles-ci avec des pathogènes bactériens à transmission vectorielle a été déterminée à l'aide du test Chi-carré (χ^2). Une valeur P inférieure à 0,05 a été considérée comme significative.

Résultats: Vingt-cinq des 80 chameaux (31,3%) étaient positifs à au moins un agent pathogène bactérien à transmission vectorielle, *Anaplasma phagocytophilum* (22,5%, 18/80) étant l'espèce la plus répandue, suivie d'*Anaplasma platys* (7,5 %, 6/80) et *Bartonella dromedarii* (2,5%, 2/80). Un seul chameau a été co-infecté par deux agents pathogènes. Tous les échantillons ont été testés négatifs pour *Rickettsia* spp et *Coxiella burnetii*. Aucun des facteurs (âge, sexe, infestation par les tiques et sites d'étude) n'était significativement associé à la prévalence des bactéries à transmission vectorielle chez les chameaux (*p*>0,05).

Conclusion: La présente étude est le premier rapport d'anaplasmose et de bartonellose chez les «*Camelus dromedaries*» d'Algérie. Nos résultats ont mis en évidence la nécessité de poursuivre les recherches sur les agents pathogènes transmis par les tiques chez les chameaux.

Mots clés: *Anaplasma phagocytophilum, Anaplasma platys, Bartonella*, bactéries transmises par les tiques, détection moléculaire, dromadaires, Algérie

Introduction:

Dromedaries are widespread throughout northern and eastern Africa. Algeria covers an area of 2,381,741 km², of which 87% is occupied by the Sahara where 381.882 camels live (1). One-humped camels "*Camelus dromedaries*" are an almost exclusively domesticated species that are common in arid areas. Many people of Sahara practice camel rearing for a livelihood. Camel breeding plays an important economic role in these areas, providing a wide diversity of goods (milk and meat, wool and skin) and services (agricultural activities, transport and tourism).

The hardiness of camels in arid regions has made humans more dependent on them. Despite close association between camels and humans, investigations on vector borne zoonotic bacterial pathogens infecting camels are scarce compared to other animals. In Algeria, most epidemiological surveys have focused on the research of trypanosomiasis in dromedaries (2-5). New data have confirmed that camels are susceptible to a wide range of pathogens, and can act as carriers or reservoirs of tick-borne bacterial diseases. Different Anaplasma species have been identified in camel populations by molecular tools such as *Anaplasma platys, Anaplasma ovis, Anaplasma phagocytophilum, Candidatus Anaplasma camelii* and novel genetic variants associated with Anaplasma strains in Iran, Morocco, Nigeria, Tunisia, Saudi Arabia, China and Pakistan (6-17).

Anaplasmosis are among emerging and potentially fatal arthropod-transmitted diseases for humans and animals (8). Camels were found to be infected with a novel *Bartonella* spp named *Bartonella dromedarii* (18, 19). However, the epidemiological and public health importance of *Candidatus Bartonella dromedarii* in camels is not clear. Bartonella species infect a wide range of domestic and wild mammals. Several *Bartonella* spp are being recognized as important zoonotic pathogens that occur worldwide with presentations that range from subclinical to severe disease (20).

Camels may also be exposed to spotted fever group (SFG) Rickettsiae because they may show a heavy burden of ixodid ticks. The occurrence of *Rickettsia* species such as *Rickettsia aeschlimannii*, *Rickettsia africae* and *Rickettsia sibirica mongolitimonae* in Hyalomma ticks collected from camels have been reported (21-24). In addition, *Rickettsia* spp and *Rickettsia aeschlimannii* DNA were detected in the blood of camelids (24,25). In Algeria, the detection of *Rickettsia aeschlimannii* and *Rickettsia africae* have been identified only in ticks of camels (26,27). *Coxiella burnetii* have been detected in Hyalomma tick species from Tunisia and Algeria, which raises the possibility of the involvement of this arthropod vector in the active diffusion of these bacteria among camels, other domestic animals and humans (28, 29). Moreover, previous molecular research indicated that both camels and ticks could be sources for Q fever in Egypt (30).

The most remarkable findings of some serological studies were the high antibody prevalence against *Rickettsia* spp, *Anaplasma* spp and specially *Coxiella burnetii* (25,31-36). In the south-eastern part of Algeria, a high seroprevalence of Q fever has been reported am-

ong healthy camels (29,37) but little is known about these diseases among camels in Algeria. Therefore, the present study aimed to investigate the occurrence of vector-borne bacteria (*Anaplasma* spp, *Rickettsia* spp, *Coxiella burnetii* and *Bartonella* spp) in camels from Algeria by molecular methods.

Materials and methods:

Study area

The study was carried out in Laghouat city, Algeria. Laghouat is located at the boundary between the high steppe plateaus and the Saharan region, 400 km from the Mediterranean coast (Fig. 1) at 751 m above sea level and at a latitude of 33°47' 59" North and a longitude of 2° 52' 59" East. It spreads over an area of 400 km^2 (38).

Study animal and sampling

A total of 80 randomly selected dromedary camels (9 males, 71 females) reared in the surroundings of the city of Laghouat served as the study animal. These one-humped camels "*Camelus dromedaries*" were apparently healthy animals belonging to 3 different herds (Fig. 1). Whole-blood samples were collected from the jugular vein of each camel using a sterile needle and immediately placed into EDTA tubes, which were transferred to an ice-box for transport, and eventually stored at -20 °C for molecular analyses.

Animal examination and data collection

All sampled animals were restrained with the help of their owners and handled humanely. Physical examination was carried out on each animal and information provided by the owners included age and sex of camels, and place of residence. The presence or absence of ectoparasites was noted.

DNA extraction

DNA was extracted from 200 µL of blood using the DNeasy Blood and Tissue Kit (Qiagen®, Hilden, Germany), according to the manufacturer's instructions. The genomic DNA was stored at -20°C under sterile conditions until used as a template in PCR assays.

PCR amplification and sequencing

To investigate the presence of vector-borne bacteria in the blood sample from camels, extracted DNA was initially tested for detection of pathogens with the real time PCR (rt-PCR) assay, and results were deemed positive if the cycle threshold (Ct) value was lower than 32. Positive samples on rt-PCR were then confirmed by conventional PCR and sequencing of the PCR products.

For *Anaplasma* spp, all samples were screened by qPCR targeting the *23S rRNA* gene, and those positive were amplified by conventional PCR and the PCR products sequenced using the *23S* standard PCR system as described elsewhere (39). For molecular detection and identification of *Bartonella*, genus-specific qPCR was based on the 16S-23S rRNA intergenic transcribed spacer (*ITS gene*). Positive samples were confirmed by standard PCR and sequencing of the partial sequence of the citrate synthase gene (*gltA*) as previously described (40). The blood was also subjected to qPCR with *Coxiella burnetii*-specific primers and probes to amplify the *IS1111* gene and *IS30A* spacers as previously described (41). Similarly, all extracted DNA were assessed for the presence of rickettsial DNA using *Rickettsia* genus-specific qPCR for spotted-fever group (SFG) Rickettsiae by targeting the *gltA* gene (RKND03 system) (41).

In all the experiments, distilled water was included as negative control. The positive
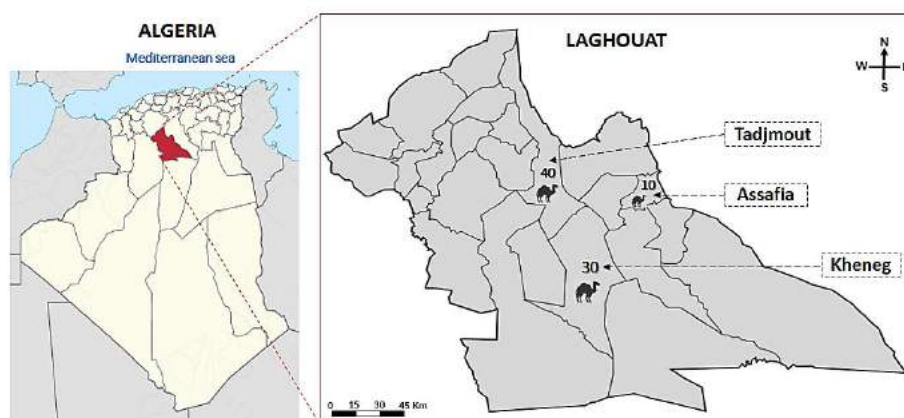

Fig. 1: A map of Laghouat city showing study sites and the number of sampled camels

controls included DNA extracted from a dilution of cultured strains of *Anaplasma phagocytophilum* (for the detection of *Anaplasma* spp), *Bartonella elizabethae* (for the detection of *Bartonella* spp), *Coxiella burnetii* (for the detection of *Coxiella burnetii*) and *Rickettsia montanensis* (for the detection of *Rickettsia* spp).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL). Association of variables (sex, age, tick infestation and location of the animals) with the prevalence of vector-borne bacterial pathogens was done using Chi-square (X^2) test. P values lower than 0.05 were considered as indicative of significance.

Results:

Following qPCR assay, 24 (30.0%) of the 80 camels were positive for *Anaplasma* spp, and 2 (2.5%) were positive for *Bartonella* spp. Only 1 (1.3%) camel was co-infected with these two agents (Table 1). All surveyed camels were negative for *Coxiella burnetii* and *Rickettsia* spp DNA. Sequencing and BLAST analyses of *Anaplasma* spp showed 100% identity with several genotypes of *Anaplasma phagocytophilum* (GenBank accession numbers CP015376.1, CP006618.1, NR_076399.1 etc) and 100% identity with *Anaplasma platys* (GenBank accession no. CP046391.1). A BLAST search of *gltA* sequences identified *Bartonella* strains which were very close to a newly proposed species, *Bartonella dromedarii* (99.59% similarity with GenBank accession numbers KJ909817.1, KJ909815.1, KJ909814.1 etc).

None of the factors (age, sex, tick infestation and location sites of camels) was significantly associated with the prevalence of vector-borne bacteria pathogens in the camel ($p>0.05$). Table 2 represents the molecular studies showing the prevalence of vector-borne bacterial pathogens in camel across the world.

Discussion:

The objective of the current study is to determine epidemiological role of camels and their zoonotic potential in the transmission of vector-borne bacteria pathogens in Algeria. In total, 31.3% of the dromedary camels studied were positive for at least one agent, including one animal co-infected with *Anaplasma platys* and *Bartonella* spp. In this study, the association between occurrence of these bacteria pathogens in the camels and selected factors (age, sex, tick infestation and study sites) was not statistically significant ($p>0.05$).

To the best of our knowledge, this study is the first report of camel anaplasmosis in Algeria. A total of 24 (30.0%) of 80 camels studied were positive to *Anaplasma* spp, 18 (22.5%) were identified as *Anaplasma phagocytophilum* and 6 (7.5%) as *Anaplasma platys*. The DNA of *A. phagocytophilum* and *A. platys* were previously identified in bovine blood samples in Algeria (39). *Anaplasma platys* infects mainly dogs, and canine infections with this agent have also been reported in Algeria by molecular methods (42,43). Furthermore, antibodies against *A. phagocytophilum* were found in dogs and horses from Algeria (43,44). In previous studies, the PCR prevalence of *A. platys* ranged from 3.33% to 61.11% among camels in many countries (13,15,45,46), and this agent was also detected in Rhipicephalus ticks collected from Bactrian camels in China (15).

Concerning *A. phagocytophilum*, very few studies have reported its occurrence in dromedaries. PCR analyses showed that 34.2%

Table 1: Prevalence of vector-borne bacterial pathogens with respect to demographic characteristics of camels

Camels	No sampled	Total of positive results	OR (or X^2)	p-value	*Anaplasma phagocytophilum*	*Anaplasma platys*	*Bartonella dromedarii*
Sex							
Male	9	4 (44.4)	1.503	0.45	1 (11.1)	*2 (22.2)	*2 (2.5)
Female	71	21 (29.5)			17 (23.9)	4 (5.6)	0
Age group (years)							
1–3	17	8 (47)	2.975[+]	0.2259	6 (35.3)	2 (11.7)	0
4–9	40	12 (30)			9 (22.5)	3 (7.5)	1 (2.5)
≥ 10	23	5 (21.7)			3 (13)	1 (4.3)	1 (4.3)
Tick infestation							
No	5	0	0.18	0.317	0	0	0
Yes	75	25 (33.3)			18 (24)	6 (8)	2 (2.5)
Study sites							
Kheneg	30	9 (30)	1.92[+]	0.3829	5 (16.6)	3 (10)	2 (6.6)
Assafia	10	5 (50)			5 (50)	0	0
Tadjmout	40	11 (27.5)			8 (20)	3 (7.5)	0
Total	**80**	**25 (31.3)**			**18 (22.5)**	**6 (7.5)**	**2 (2.5)**

*One camel coinfected by *Anaplasma platys* and *Bartonella dromedarii.*; OR=Odds ratio; [+] = Chi-square (X^2)

Table 2: Prevalence of vector-borne bacterial pathogens in camel from molecular studies around the world

Pathogens	Country	Number of animal tested	Number of animal positive	Prevalence (%)	Molecular assay used	Reference
***Anaplasma* spp**						
(*Candidatus* Anaplasma Camelii)	Saudi Arabia	100	26	26.0	PCR/Sequencing	16
	Iran	100	6	6.0	PCR/Sequencing	50
	Morocco	106	42	39.6	PCR/Sequencing	14
	Iran	200	30	15.0	PCR/Sequencing	8
	Kenya	249	171	68.7	PCR/high-resolution melting (PCR-HRM)/Sequencing	51
	Nigeria	176	71	40.3	PCR/Sequencing	52
	Kenya	296	233	78.7	PCR/high-resolution melting (PCR-HRM)/Sequencing	53
Anaplasma platys	China	279	20	7.2	PCR/Sequencing	15
	Nigeria	36	22	61.1	PCR/reverse line blotting (PCR-RLB)	13
	Saudi Arabia	170	9	5.3	PCR/Sequencing	45
	Iran	60	2	3.3	PCR/Sequencing	46
Novel *Anaplasma* sp. strains genetically related to *A. platys*	Tunisia	226	40	17.7	PCR/Sequencing	17
Anaplasma phagocytophilum	Iran	207	71	34.2	Polymerase chain reaction (PCR)/nested PCR	11
	Saudi Arabia	170	1	0.6	PCR/Sequencing	45
Anaplasma ovis	Tunisia	412	5	1.2	PCR/Sequencing	6
Novel Genetic Variants Associated to *Anaplasma ovis*	Iran	100	2	2.0	PCR/Sequencing	10
***Bartonella* spp**						
Bartonella henselae	Tunisia	412	15	3.6	PCR/Sequencing	48
(*Candidatus* Bartonella dromedarii)	Iran	106	18	17.0	PCR/Sequencing	19
***Rickettsia* spp**						
R. africae	Egypt	61	25	41.0	PCR/Sequencing	54
Rickettsial species	Tunisia	293	8	2.7	PCR/Sequencing	24
R. aeschlimannii			2	0.7		
R. monacensis			4	1.4		
R. helvetica			1	0.3		
R. massiliae			1	0.3		
R. africae			1	0.3		
Coxiella burnetii						
	Iran	130	14	10.8	PCR	55
	Saudi Arabia	82	13	15.9	PCR	56
	Egypt	113	52	46.0	PCR/Sequencing	30
	Tunisia	412	0	0	PCR	28
	Algeria	184	0	0	qPCR	29
	Kenya	296	10	3.4	PCR/high-resolution melting (HRM) analysis/Sequencing	53

Vector-borne bacterial pathogens in dromedary camels

Afr. J. Clin. Exper. Microbiol. 2022; 23 (2): xxxx

(71/207) of camels harbored *A. phagocytophilum* in Iran (11). More recently, DNA of this pathogen was identified in only one camel among 170 tested (0.6%) in Saudi Arabia (45). In Tunisia, seropositivity to *A. phagocytophilum* was detected in camels by indirect immunofluorescence (IFA) (12). *Anaplasma phagocytophilum* is an emerging tick-borne zoonotic pathogen of increased interest worldwide which has medical as well as veterinary importance. This bacterium is the causative agent of human granulocytic anaplasmosis associated with high mortality in humans. *Anaplasma platys* could also possibly have a zoonotic potential (47). Anaplasmosis has been described to be a subclinical disease in Arabian one-humped camels (*Camelus dromedarius*) (15). The presence of dogs in the camel farms explains the positivity of the camels to anaplasmosis in our study. In addition, all of the positive camels in our study harbored ticks. Therefore, they can be directly affected by tick-borne pathogens and can play the role of reservoir for further transmission to humans and other animals. New species of *Anaplasma* have been identified infecting dromedaries in other studies (8,14,16).

The DNA of *Bartonella* spp was found in only two camels with a prevalence of 2.5%. Sequence comparisons analysis showed a high homology (99.59%) to *Bartonella dromedarii*. In a previous study, Rasis and colleagues (18) confirmed the presence of a novel species of *Bartonella* in camelids, which has been named *Bartonella dromedarii* sp. Nov. Lately, *Candidatus Bartonella dromedarii* was identified in camels from Iran (19) with a prevalence of 17% (18/106). More studies are required to evaluate precisely the risk factors, transmission routes, and the ability to infect humans of this *Bartonella* species. Other studies reported the presence of two species of *Bartonella* (*B. rochalimae* and *B. bovis*) in *Hyalomma dromedarii* ticks collected from camels (20). A recent study reported the occurrence of novel *B. henselae* genotypes closely related to those isolated from humans in Tunisian dromedaries (48). The identification of new *Bartonella* variants in camels suggests a continuous evolution of strains' diversity which is related to a complex maintenance of this bacterium in nature, as was observed in other mammals (49). The camels in our study could have been in contact with wild canids and felids as well as rodents which are the animals most frequently found in the Algerian desert.

In the present study, all tested samples yielded negative results for *Rickettsia* spp and *Coxiella burnetii*. Nevertheless, these findings do not exclude the presence of these agents in camels since we worked on a small sample and in only one region. Limited resources, low levels of regulation, poor hygiene,

high mobility of animals and herders, heavy infestation by ectoparasites, the close human to camel contact and lack of consistent veterinary care can justify the probable role of camelids as a significant source for zoonotic diseases in Algeria.

Conclusion:

We report here the first molecular detection of *Anaplasma* spp and *Bartonella* spp in dromedary camels suggesting a possible involvement of camelids as hosts or reservoirs in the transmission cycle of these agents in arid and Saharan areas in Algeria. Therefore, further extensive molecular surveys on large number of samples covering many localities in the country are needed in order to correctly address the prevalence and geographical distribution of vector-borne and zoonotic diseases in camels. This in turn will help in designing and implementation of effective preventive and control measures.

Acknowledgements:

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Short Communication   Open Access

Assessment of selected liver enzyme activity in patients with rifampicin-resistant tuberculosis receiving treatment at a tertiary healthcare facility, southwest Nigeria

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Abstract:

Background: Several anti-tuberculous drugs have been effective in the treatment and management of drug-sensitive and -resistant tuberculosis (TB). While these drug combinations have proven to be highly active against tubercle bacilli, side effects and toxicity may occur with tendency to interrupt or discontinue therapy, resulting in poor compliance. The objective of this study is to assess hepatotoxic potentials of anti-TB drugs among patients with rifampicin-resistant TB (RRTB) undergoing treatment at the directly observed treatment short-course (DOTS) clinic of Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Ogbomoso, Nigeria.

Methodology: This was a prospective study of 40 patients with RRTB on second-line anti-TB therapy including bedaquiline, moxifloxacin, prothionamide, ethambutol, pyrazinamide, isoniazid and clofazimine. RRTB was diagnosed by sputum smear AFB microscopy and Xpert MTB/RIF assay at the TB laboratory of Bowen University Teaching Hospital, Ogbomoso, Nigeria. Forty gender and age-matched apparently healthy persons were used as control. Venous samples (~5ml) were collected from each participant at baseline (prior to commencement of anti-TB therapy) and after completion of 9-11 months therapy, as well as from the controls. Plasma was separated by centrifugation and the activity of ALT, AST and ALP was measured by spectrophotometric analysis, while total protein and albumin levels were determined using routine methods. Data were presented as mean±SD and analysed using SPSS version 21.0. Comparison of the mean enzyme activity at baseline and after completion of therapy as well as with the control was done with unpaired 't' test, and 'p' (two tail) value less than 0.05 was considered statistically significant.

Results: The age range of the 40 RRTB patients is 20-67 years (mean age 45.50±10.1 years) while the age range of the 40 controls is 21-65 years (mean age 45.70±12.10 years). The male to female ratio is 1.2:1 for the patients and 1:1 for the control. There is statistically significant increase in post-therapy plasma activity of ALT ($p<0.0001$), AST ($p<0.0001$), ALP ($p<0.0001$), and total protein level ($p=0.0086$) compared to the baseline, while plasma albumin level decreased significantly post-therapy ($p=0.007$). Although there is no significant difference in the baseline activity of ALT ($p=0.4936$) and AST ($p=0.2539$) for the RRTB patients compared to the control, post-treatment activity of ALT ($p<0.0001$) and AST ($p<0.0001$) in RRTB patients were significantly higher than in apparently heathy controls.

Conclusion: The activity of the liver enzymes (AST and ALT) reported among RRTB patients in our study are within the normal reference range for persons above 18 years of age, indicating a non-hepatotoxic effect of the anti-TB drugs. However, statistically significant increase in these enzyme activities in the patients' post-treatment compared to the baseline, and to apparently healthy controls, indicates that the drugs may be potentially hepatotoxic on prolonged usage.

Keywords: rifampicin resistant tuberculosis; anti-TB drugs; ALT; AST; ALP; hepatotoxicity

Évaluation de l'activité des enzymes hépatiques sélectionnées chez les patients atteints de tuberculose résistante à la rifampicine recevant un traitement dans un établissement de soins de santé tertiaires, dans le sud-ouest du Nigeria

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Résumé:

Contexte: Plusieurs médicaments antituberculeux se sont révélés efficaces dans le traitement et la prise en charge de la tuberculose pharmacosensible et résistante. Bien que ces combinaisons de médicaments se soient avérées très actives contre les bacilles tuberculeux, des effets secondaires et une toxicité peuvent survenir avec une tendance à interrompre ou à interrompre le traitement, entraînant une mauvaise observance. L'objectif de cette étude est d'évaluer les potentiels hépatotoxiques des médicaments antituberculeux chez les patients atteints de tuberculose résistante à la rifampicine (RRTB) qui suivent un traitement à la clinique DOTS (Traitement de courte durée directement observé) de l'Université de technologie de Ladoke Akintola (LAUTECH), Hôpital, Ogbomoso, Nigéria

Méthodologie: Il s'agissait d'une étude prospective de 40 patients atteints de RRTB sous traitement antituberculeux de deuxième ligne comprenant la bédaquiline, la moxifloxacine, le prothionamide, l'éthambutol, le pyrazinamide, l'isoniazide et la clofazimine. La RRTB a été diagnostiquée par microscopie AFB des frottis d'expectoration et test Xpert MTB/RIF au laboratoire de la tuberculose de l'hôpital universitaire de Bowen, à Ogbomoso, au Nigeria. Quarante personnes apparemment en bonne santé appariées selon le sexe et l'âge ont été utilisées comme contrôle. Des échantillons veineux (~5ml) ont été prélevés sur chaque participant au départ (avant le début du traitement antituberculeux) et après la fin du traitement de 9 à 11 mois, ainsi que sur les témoins. Le plasma a été séparé par centrifugation et l'activité de l'ALT, de l'AST et de l'ALP a été mesurée par analyse spectrophotométrique, tandis que les taux de protéines totales et d'albumine ont été déterminés à l'aide de méthodes de routine. Les données ont été présentées sous forme de moyenne ± ET et analysées à l'aide de SPSS version 21.0. La comparaison de l'activité enzymatique moyenne au départ et après la fin du traitement ainsi qu'avec le contrôle a été effectuée avec un test «*t*» non apparié, et une valeur «*p*» (deux queues) inférieure à 0,05 a été considérée comme statistiquement significative.

Résultats: La tranche d'âge des 40 patients RRTB est de 20 à 67 ans (âge moyen 45,50±10,1 ans) tandis que la tranche d'âge des 40 témoins est de 21 à 65 ans (âge moyen 45,70±12,10 ans). Le ratio hommes/femmes est 1.2:1 pour les patients et 1:1 pour le contrôle. Il y a une augmentation statistiquement significative de l'activité plasmatique post-thérapie de l'ALT (*p*<0,0001), de l'AST (*p*<0,0001), de l'ALP (*p*<0,0001) et du taux de protéines totales (*p*=0,0086) par rapport à la ligne de base, tandis que l'albumine plasmatique le niveau a diminué significativement après le traitement (*p*=0,007). Bien qu'il n'y ait pas de différence significative dans l'activité de base de l'ALT (*p*=0,4936) et de l'AST (*p*=0,2539) pour les patients atteints de RRTB par rapport au groupe témoin, l'activité post-traitement de l'ALT (*p*<0,0001) et de l'AST (*p*<0,0001) chez les patients RRTB étaient significativement plus élevés que chez les témoins apparemment en bonne santé.

Conclusion: L'activité des enzymes hépatiques (AST et ALT) rapportée chez les patients atteints de RRTB dans notre étude se situe dans la plage de référence normale pour les personnes de plus de 18 ans, indiquant un effet non hépatotoxique des médicaments antituberculeux. Cependant, une augmentation statistiquement significative de ces activités enzymatiques chez les patients après le traitement par rapport à la ligne de base et à des témoins apparemment sains, indique que les médicaments peuvent être potentiellement hépatotoxiques en cas d'utilisation prolongée.

Mots clés: tuberculose résistante à la rifampicine; médicaments antituberculeux; ALT; AST; ALP; hépatotoxicité

Introduction:

Tuberculosis remains a global public health concern, and a leading cause of death worldwide, particularly in developing countries (1). The causative pathogen, which are members of the *Mycobacterium tuberculosis* complex (MTBC), primarily affects the lungs causing pulmonary TB, but can also affect other organs outside the lungs (extra-pulmonary TB). Members of MTBC include *Mycobacterium tuberculosis*, *Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti,* and *Mycobacterium canetti* (2). The burden of TB remains high globally with an estimated 10 million new cases and 1.5 million TB-related deaths in 2019 (3), however, case detection rate is only about 64%, with estimated 36% undetected. Nigeria is

ranked seventh among the 30 high-TB burden countries and second in Africa, with an estimated 407,000 TB cases and 154,000 deaths per year (4). Management of TB has been made worse in Nigeria by issues of drug resistance, HIV/AIDS epidemic, and low treatment coverage resulting from low TB case finding for both adults and children (5).

The first line drugs for the treatment of drug-sensitive TB includes rifampicin, pyrazinamide, ethambutol, and isoniazid, while second line drugs are reserved for drug resistant TB, and includes drugs in different classes such as fluoroquinolones (levofloxacin, moxifloxacin, gatifloxacin), linezolid, cycloserine, bedaquiline, delamanid, imipenem, ethionamide, para-amino salicylic acid, clofazimine, injectable aminoglycosides among others (6). There has however been increase

in resistance of tubercle bacilli to these drugs (7) with emergence of multi-drug resistant (MDR) strains.

Multidrug resistance is defined as resistance to both isoniazid and rifampicin, with or without resistance to other anti-TB drugs, and is classified into two categories; resistance to only isoniazid and rifampicin, known as basic MDR-TB, and resistance to these two drugs, in addition to one or more first and/or second-line drugs, categorized as MDR-TB-plus (8). Rifampicin resistant TB (RRTB) is defined by the World Health Organization (WHO) as any resistance to rifampicin including mono-resistance, multidrug resistance, extensively drug resistance and pandrug resistance (6). When compared to first-line treatment, RRTB treatment is generally extended and involves the use of less effective drug regimens with more adverse effects (9). In a systematic review and meta-analysis of prevalence of TB in Nigeria, Oyedum and his colleagues (10) reported resistant rates to any TB drug among new and previously treated TB cases of 32% and 53%, and MDR rates of 6% and 32% respectively.

Minor adverse effects of second-line anti-TB drugs are quite common but can be easily managed. However, some adverse effects such as nephrotoxicity from injectable aminoglycosides, cardiotoxicity from fluoroquinolones, hepatic, intestinal, and central nervous toxicity, can be life-threatening (11). Liver enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) are commonly used to assess liver functions and determine hepatocellular damage. Hepatotoxicity has been associated with an increase in serum ALT and AST greater than three times of upper limit of reference (ULR). The objective of this prospective study is to assess the potential risk of drug-induced liver injury in a cohort of patients with RRTB on second-line anti-TB drugs at the DOTS clinic of LAUTECH Teaching Hospital, Ogbomoso, Nigeria, by assay of plasma activity of ALT, AST and ALP as well as serum levels of total protein and albumin in these patients (pre-and post-treatment), and comparing these with those from apparently healthy individuals.

Materials and method:

Study setting

This study was carried out at the directly observed treatment short-course (DOTS) clinic of Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Ogbomoso, Oyo State, southwest Nigeria. The State has an estimated population of 5,580,894 (12) and a land mass of 28,454 square kilometers with an equatorial climate of dry and wet seasons of relatively high humidity. Ogbomoso is the second largest city in Oyo State.

Study design and subject participants

This was a prospective study of 40 rifampicin-resistant TB (RRTB) patients on second line anti-TB therapy for a duration of 9-11 months (depending on compliance and sputum conversion) who were randomly selected among TB patients attending the DOTS clinic or hospitalized between January 2019 and July 2021. The second line drugs used included WHO approved combination regimen for RRTB (13), with bedaquiline, clofazimine, prothionamide, pyrazinamide, ethambutol and high-dose isoniazid for the intensive phase of 6 months followed by moxifloxacin, clofazimine, pyrazinamide and ethambutol for the continuation phase of 5 months. Forty apparently healthy adults were randomly recruited among voluntary blood donors within the period of the study to serve as control.

Ethical approval

Ethical approval was obtained from the Ethical Review committee of LAUTECH Teaching Hospital. Written informed consent was obtained from all participants and confidentiality of data was assured.

Inclusion and exclusion criteria

The criteria for inclusion in the study were; (i) written informed consent of participants, (ii) fulfilment of clinical and laboratory criteria for diagnosis of RRTB based on radiological evidence of TB, sputum smear positive for AFB on Ziehl-Neelsen (ZN) stain, and positive Xpert MTB/RIF assay, and (iii) willingness to comply with anti-TB treatment regimen. Exclusion criteria were patients already on second line anti-TB drugs before recruitment, pregnancy, co-infection with HIV, patients with features of acute and chronic liver diseases, and patients positive for hepatitis B and C viruses.

Data and samples collection

Baseline demographic data (age and gender) of each selected participant were collected into a designed data collection form before commencement of second line anti-TB drugs. Sputum samples (~10 ml) were collected into sterile universal bottles from each study participant according to the DOTS method of spot, morning and spot (SMS) protocol for the purpose of AFB microscopy by ZN stain and Xpert MTB/RIF assay. Approximately 5ml of venous blood were also collected from each patient and control into ethylene diamine tetra-acetic acid (EDTA) specimen bottles for liver enzyme analysis. At the completion of anti-TB therapy, another

5ml of blood samples were collected from the patient to measure the activity of the liver enzymes.

Sample processing and enzyme activity measurement

Microscopic examination for AFB by ZN stain was carried out by two trained medical laboratory scientists. Only concordant positive results were considered in line with WHO criteria for smear positive TB (14). RRTB was detected by Xpert MTB/RIF assay at the TB laboratory of Bowen University Teaching Hospital, Ogbomoso, Nigeria.

AST activity (measured in IU/L) was assayed by spectrophotometric measurement of the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyldrazine while ALT activity was determined by measuring the concentration of pyruvate hydrazone formed with 2,4-dinitro phenyldrazine according to Reitman and Frankel (15), using analytical grade commercial reagents (Randox Laboratories Limited, United Kingdom) that can assay between 7 to 89 IU/L and 4 to 94 IU/L activity of AST and ALT respectively. ALP activity was determined by measuring the concentration of chromogen formed from the enzyme activity on 2-amino-2-methyl-1-propanol-buffered sodium thymolphthalein monophosphate with commercial assay kit (Teco Diagnostics, Anaheim, USA) that has detection limits of 1 to 100 IU/L. Total plasma protein was estimated using Biuret method (16) while plasma albumin was measured by the routine method of Tietz (17).

Statistical analysis

Data were presented as mean±SD and analysed with the Statistical Package for the Social Sciences (SPSS) version 21.0. Difference in mean values of enzyme activity between pre- and post-TB treatment, and with apparently healthy control was determined using unpaired 't' test and 'p' value (two-tail) less than 0.05 was considered as statistically significant.

Results:

A total of 40 rifampicin-resistant TB patients (age range 20-67 years, mean age 45.50±10.1 years) and 40 gender and age-matched apparently healthy individuals (age range 21-65 years, mean age 45.70 ±12.10 years) were enrolled for the study (Table 1). The male to female ratio in the patients is 1.2:1 while the male to female ratio in the controls is 1:1.

Table 2 is a comparison of liver parameters pre- and post-TB therapy which showed statistically significant increase in post-therapy plasma activity of ALT (95% CI = 2.439-5.361, $p<0.0001$), AST (95% CI = 7.471-10.729, $p<0.0001$) and ALP (95% CI =2.846-7.354, $p<0.0001$) over the baseline values. The plasma total protein level was also significantly higher post-therapy (95% CI=1.463 to 9.637, $p=0.0086$), while plasma albumin level decreased significantly post-therapy (95% CI= -6.69 to -1.11, $p=0.007$).

Table 3 is a comparison of liver enzyme activity of patients with RRTB at baseline (prior to anti-TB therapy commencement) with apparently healthy controls, which showed that there is no significant difference in the baseline activity of ALT (95% CI = -0.569 to +1.169, $p=0.4936$) and AST (95% CI = -0.659 to +2.459, $p=0.2539$) of the patients and the control, but ALP activity was significantly higher in the control than baseline activity in RRTB patients (95% CI=3.936 to 7.864, $p <0.0001$).

Table 4 is a comparison of liver enzyme activity of patients with RRTB post-treatment with apparently healthy controls, which showed that post-treatment ALT (95% CI = 2.126 to 5.074, $p<0.0001$) and AST (95% CI= 6.520 to 9.871, $p<0.0001$) activity were significantly higher in RRTB patients' than apparently heathy controls, but no significant difference in the activity of ALP between the two groups (95% CI = -2.937 to 1.337, $p=0.4579$)

Table 1: Gender and age distribution of selected patients with rifampicin resistant tuberculosis and apparently healthy controls in LAUTECH Teaching Hospital, Ogbomoso, Nigeria

Demographic variable	RRTB patient (n=40)		Apparently healthy control (n=40)	
Age groups (years)	Male	Female	Male	Female
20-29	2	2	2	2
30-39	2	3	5	4
40-49	9	8	2	7
50-59	7	3	6	5
≥60	2	2	5	2
Total	**22**	**18**	**20**	**20**
Mean age	45.50 ± 10.1		45.70 ±12.10	
Age range	20 – 67		21 – 65	
Male: Female ratio	1.2: 1		1: 1	

RRTB = rifampicin resistant tuberculosis; n=number

Table 2: Comparison of liver parameters before and after anti-tuberculous treatment in patients with rifampicin-resistant tuberculosis in LAUTECH Teaching Hospital, Ogbomoso, Nigeria

Liver parameters	Values at baseline (mean±SD)	Values post-treatment (mean±SD)	95% CI	p value
Alkaline phosphatase (IU/L)	15.1 ± 4.7	20.2 ± 5.4	2.846 to 7.354	<0.0001*
Aspartate transaminase (IU/L)	7.5 ±3.4	16.6 ± 3.9	7.471 to 10.728	<0.0001*
Alanine transaminase (IU/L)	4.70 ±1.9	8.6 ±4.2	2.439 to 5.361	<0.0001*
Total protein (g/dL)	71.25±6.80	76.8±11.01	1.463 to 9.637	0.0086*
Albumin (g/dL)	42.1±3.78	38.2±7.95	-6.689 to -1.111	0.0070*

*p value (two-tail) < 0.05 was taken as statistically significant; reference range: ALP 30-120 IU/L; AST 0-35 IU/L; ALT 0-45 IU/L; total protein 60-83 g/dL; albumin 35-50 g/dL; SD = standard deviation; CI = confidence interval

Table 3: Comparison of liver enzyme activity of patients with rifampicin resistant tuberculosis at baseline with apparently healthy controls in LAUTECH Teaching Hospital, Ogbomoso, Nigeria

Liver enzyme activity	Values at baseline (mean± SD)	Values in healthy controls (mean ±SD)	95% CI	p value
Alkaline phosphatase (IU/L)	15.1 ± 4.7	21.00 ±04.10	3.936 to 7.864	<0.0001*
Aspartate transaminase (IU/L)	7.5 ±3.4	08.40 ± 03.60	-0.6590 to +2.459	0.2539
Alanine transaminase (IU/L)	4.70 ±1.9	05.00 ±02.00	-0.5685 to +1.169	0.4936

*p value (two-tail) < 0.05 was taken as statistically significant; reference range: ALP 30-120 IU/L; AST 0-35 IU/L; ALT 0-45 IU/L; SD = standard deviation; CI = confidence interval

Table 4: Comparison of liver enzyme activity of patients with rifampicin resistant tuberculosis post-treatment with apparently healthy controls in LAUTECH Teaching Hospital, Ogbomoso, Nigeria

Liver enzyme activity	Values post treatment (mean± SD)	Values in healthy controls (mean ±SD)	95% CI	p value
Alkaline phosphatase (IU/L)	20.2 ± 5.4	21.00 ±04.10	-2.937 to 1.337	0.4579
Aspartate transaminase (IU/L)	16.6 ± 3.9	08.40 ± 03.60	6.529 to 9.871	<0.0001*
Alanine transaminase (IU/L)	8.6 ±4.2	05.00 ±02.00	2.126 to 5.074	<0.0001*

*p value (two-tail) < 0.05 was taken as statistically significant; reference range: ALP 30-120 IU/L; AST 0-35 IU/L; ALT 0-45 IU/L; SD = Standard deviation; CI = confidence interval

Discussion:

The mean age of the patients with tuberculosis in our study falls within the age group reported by Dharmik and his collea-gues (18), who ascribed the high prevalence of TB in this age group to increased physical activity, exposure and socio-economic factors as predisposing factors to the disease. Glo-bally, MDRTB/RRTB is rapidly becoming a major concern in the care, treatment and management of TB, which have emerged through inappropriate use of first line anti-TB drugs especially isoniazid, rifampicin and ethambutol. Meanwhile, anti-TB drug-induced hepatocellular injury have been widely repor-ted (18,19,20,21), and Enoh et al., (21) reported hepatotoxicity to be more prevalent in patients over 40 years of age. Adverse effects of drugs could alter treatment and results in poor outcomes from poor compli-ance with therapy. Our study revealed that plasma activity of aminotransferases (AST and ALT) increased significantly among RRTB patients after treatment with the standard second line anti-TB (bedaquiline, isoniazid, levofloxacin, linezolid and clofazimine) used

in our center, which agree with the study of Mirlohi et al., (20) that reported increased activity of AST and ALT among MDR-TB patients on anti-TB drugs.

Hepatotoxicity is usually associated with increased enzyme activity three times of upper limit of reference (ULR), even as reference values provide the basis for inter-pretations of results in clinical laboratory. As the reference range for AST and ALT activity is 0-35 IU/L and 0-45 IU/L respectively for individuals above 18 years of age (22), the enzyme activity in our study did not confirm hepatotoxicity in the patients as the enzyme activity were within normal reference range and not up to three times of ULR. However, statistically significant increase in enzyme activity in the patients' post-treatment when compared with the baseline and to the activity in apparently healthy controls, indi-cates that the drugs may be potentially hepatotoxic on prolonged usage. This is supported by the finding of significantly decreased albumin level post-therapy com-pared to baseline, which indicate that synthe-tic function of the liver may be potentially affected by the drugs

On the other hand, ALP has wide-spread tissue distribution including liver, bone, placenta and gastrointestinal tract, and its activity level have been found useful as a marker of hepatic cholestasis (23), but less useful as a marker of hepatocellular injury. In our study, ALP activity was also within the normal reference range (30-120 IU/L) pre- and post-TB therapy. Infact, the activity was significantly higher in healthy controls compared to RRTB patients at baseline, while there was no significant difference in the activity between RRTB patients' post-therapy and the controls.

Conclusion:

Our study showed the activity of liver enzymes (AST, ALT and ALP) in RRTB patients on second-line anti-TB drugs to be within the normal reference range for individuals above 18 years of age, indicating a non-toxic effect of these anti-TB drugs on the liver. However, statistically significant increase in post-treatment activity of AST and ALT, and significant decrease in albumin level in our patients compared to their baseline levels is an indication that the drugs may be potentially hepatotoxic on prolonged usage. We therefore recommend short term treatment regimen for RRTB patients, and close monitoring of hepatic functions of the patients during therapy. Further study will be required to evaluate hepatoxic potential of each of the second line anti-TB drugs.

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Authors' contributions:

OOA, OAK and AAA were involved in the study design, data collection, literature review, and manuscript draft preparation; OJO, ZTO, ATW, OAM and OAM were involved in data collection and literature review; and TSS was involved in critical review and correction of the draft manuscript and statistical analysis. All authors read and agreed with the final manuscript submitted.

Conflict of interest:

No conflict of interest is declared

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Short Communication　　　　　　　　　　　　　　　　　　**Open Access**

Infectious diseases co-morbidities among patients attending Kogi State University Teaching Hospital: a ten-year retrospective study

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Abstract:

Background: Co-existence of more than one acute or chronic infectious diseases in a person either concurrently or sequentially with consequent economic burden varies differently from one part of the world to another, with regional and population specific patterns. This study aims to provide co-morbid patho-epidemiological pattern of six infectious diseases; HIV, tuberculosis (TB), malaria, syphilis, hepatitis B and hepatitis C virus infections.
Methodology: This research is a ten-year retrospective review of records of patients admitted at various wards of Kogi State University Teaching hospital and referred to the Laboratory Department of the hospital for investigations between June 2012 and July 2021. HIV was screened using the national serial algorithm, TB was diagnosed with the GeneXpert MTB, malaria parasite was identified by blood film microscopy, and syphilis, hepatitis B and hepatitis C viruses were screened using rapid diagnostic kits. Data were analysed with SPSS version 23.0 and association of variables with respect to gender and age group was determined using Chi square, with $p< 0.05$ considered to be statistically significant.
Results: A total of 223 patients were retrospectively reviewed with 102 (45.7%) males and 121 (54.3%) females. Co-morbidities occurred most frequently among age groups 21-30 years (34.1%), 31-40 years (39.0%) and 41-50 years (16.6%). The most frequent co-morbidity pattern was HIV/TB (81.6%) followed by HBV/MP (4.5%), HIV/HBV (4.0%), HIV/MP (3.1%), TB/MP (2.7%), HBV/HCV (2.2%) while HCV/MP, TB/HCV, HCV/syphilis, and TB/HBV were (0.4%) each. There was no significant difference in the frequency of co-morbidity with respect to gender and age groups of patients ($p>0.05$).
Conclusion: Co-existence of chronic infectious disease in a person increases the risk of morbidities and mortalities. Therefore, diagnosis, and concurrent treatment and management of co-morbid infectious diseases should be incorporated into our routine healthcare system and appropriate resources should be allotted for this in health plans.

Keywords: co-morbidity, retrospective, patho-epidemiological, infectious diseases

Co-morbidités des maladies infectieuses chez les patients fréquentant l'hôpital universitaire de l'État de Kogi: une étude rétrospective sur dix ans

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Résumé:

Contexte: La coexistence de plusieurs maladies infectieuses aiguës ou chroniques chez une personne, simultanément ou séquentiellement, avec un fardeau économique conséquent, varie différemment d'une partie du monde à l'autre, avec des schémas régionaux et spécifiques à la population. Cette étude vise à fournir le schéma patho-épidémiologique comorbide de six maladies infectieuses; VIH, tuberculose (TB), paludisme, syphilis, infections par le virus de l'hépatite B et de l'hépatite C.

Méthodologie: Cette recherche est un examen rétrospectif sur dix ans des dossiers de patients admis dans divers services de l'hôpital universitaire de l'État de Kogi et référés au département de laboratoire de l'hôpital pour des enquêtes entre juin 2012 et juillet 2021. Le VIH a été dépisté à l'aide de la série nationale algorithme, la tuberculose a été diagnostiquée avec le GeneXpert MTB, le parasite du paludisme a été identifié par microscopie de frottis sanguin et les virus de la syphilis, de l'hépatite B et de l'hépatite C ont été dépistés à l'aide de kits de diagnostic rapide. Les données ont été analysées avec SPSS version 23.0 et l'association des variables par rapport au sexe et au groupe d'âge a été déterminée à l'aide du Chi carré, avec $p<0,05$ considéré comme statistiquement significatif.

Résultats: Un total de 223 patients ont été revus rétrospectivement avec 102 (45,7%) hommes et 121 (54,3%) femmes. Les comorbidités sont survenues le plus fréquemment dans les groupes d'âge 21-30 ans (34,1 %), 31-40 ans (39,0%) et 41-50 ans (16,6%). Le schéma de comorbidité le plus fréquent était le VIH/TB (81,6%), suivi du VHB/MP (4,5%), du VIH/VHB (4,0%), du VIH/MP (3,1%), de la TB/MP (2,7%), du VHB/VHC (2,2%) tandis que VHC/MP, TB/VHC, VHC/syphilis et TB/VHB étaient (0,4%) chacun. Il n'y avait pas de différence significative dans la fréquence des comorbidités en fonction du sexe et des tranches d'âge des patients ($p>0,05$).

Conclusion: La coexistence de maladies infectieuses chroniques chez une personne augmente le risque de morbidité et de mortalité. Par conséquent, le diagnostic, le traitement et la gestion concomitants des maladies infectieuses comorbides doivent être intégrés à notre système de soins de santé de routine et des ressources appropriées doivent être allouées à cet effet dans les plans de santé.

Mots-clés: comorbidité, rétrospective, patho-épidémiologique, maladies infectieuses

Introduction:

Infectious disease is a state in which pathogens such as bacterial, virus, fungi and parasite cause sufficient tissue damage while living and reproducing in host tissues (1). The concept of co-morbidity is the co-existence of more than one acute or chronic disease in one person either concurrently or sequentially. People living with acute or chronic primary infectious diseases are at higher risk of developing secondary infections (2). The global burden of co-existing infectious diseases, in particular human immunodeficiency virus (HIV), hepatitis B and C, tuberculosis (TB), malaria and syphilis in a person either concurrently or sequentially, accounts for quarter to a third of deaths worldwide (3). According to the WHO report of 2004 (4), children under five years of age account for over half of these mortalities with the fastest increase recorded in low and middle-income countries, particularly the sub-Saharan Africa.

A population-based study indicated that many tropical infectious diseases exhibit common epidemiological characteristics with age and shared mutual risk factors including poor sanitation and socioeconomic lifestyles that enhances the co-existence of these organisms in the same person with resulted concomitant morbidity (5). Viral hepatitis is one of the leading disease responsible for morbidity and mortality among persons infected with HIV (6). Of 33 million people living with HIV globally, 5-20% are co-infected with hepatitis B virus and 5-15% with hepatitis C, notwithstanding, the percentage may be up to 90% among drug addicts who inject drugs (7). Global emergencies of TB and HIV/AIDS co-infection are the major cause of death among people living with HIV (8). The morbid interaction between these two chronic diseases accounts for 25% of HIV-associated deaths globally and has increased the burden of tuberculosis by twenty to forty-fold with the greatest global burden of HIV-related TB epidemic in sub-Saharan Africa (9).

Syphilis and other infectious diseases are often co-morbid; most of these co-exist chronic infectious diseases are asymptomatic. However, even asymptomatic infectious diseases can cause complications by suppressing the infected host immune systems (10). Although there are considerable studies on hepatitis B/C, HIV/TB, HIV/hepatitis B, HIV/hepatitis C and HIV/malaria co-morbidity and co-infections, there is distinctly fewer reports of clinical and immunological morbid interactions between TB/malaria, syphilis/malaria and other related infe-

Retrospective study of infectious diseases co-morbidities

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ctious diseases. The epidemiological links between chronic infectious diseases are not well established, unlike HIV/AIDS, hepatitis B and C, and syphilis. Tuberculosis and malaria are ancient infectious diseases that have molecular evidence of co-existence, and co-evolved in the same human populations over millennia as demonstrated in mummified remains of 2000-3000 years old from Lower Egypt (11).

For this study, we focused on close co-morbid relationship between six common chronic infectious diseases in this geopolitical area, namely HIV/AIDS, TB, syphilis, malaria, hepatitis B and C viral infections, with the aim of providing co-morbid patho-epidemiological pattern that may be selectively used for epidemiological control and therapeutic management.

Materials and method:

Study design and subjects

This study is a ten-year retrospective review of records of patients admitted at various wards of Kogi University Teaching Hospital and referred to the Medical Laboratory Department for investigations of suspected HIV, tuberculosis, syphilis, malaria, hepatitis B and C virus infections from June 2012 to July 2021. The Research and Ethical Committee of the Kogi State University Teaching Hospital (KSUTH) approved the study.

Study population and data collection

A total of two hundred and twenty-three (223) patients referred to the Medical Laboratory Department of the hospital for investigations on suspicion of different infective conditions, who were confirmed to have concurrent or sequential co-infections of HIV, TB, syphilis, malaria, hepatitis B and hepatitis C, were studied. A designed data collection proforma was used to retrieve demographic information (age and gender) of each participant, detail of the tests performed and the results from laboratory register. The laboratory investigation methods used during the period of the study included; serology for HIV detection that was interpreted based on the national serial algorithm, TB was detected by the GeneXpert

MTB, malaria parasite was detected by blood film microscopy, and syphilis, hepatitis B and C virus infections were screened using LabACON rapid diagnostic kits.

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 23.0. Frequency distribution tables were generated while categorical variables were compared using Chi square (X^2) test to determine association of co-morbidity with respect to gender and age groups of patients. A p-value less 0.05 was considered as statistically significant.

Results:

A total of 223 patients with co-morbid infections were retrospectively studied, with mean age of 34.89±9.88 years, median age of 35.0 years, modal age of 30.0 years, and age range of 8-72 years. The gender distribution showed that 45.7% (102/223) were males while 54.3% (121/223) were females (Table 1). The most frequent co-infection was HIV/TB (81.6%, 182/223) followed by HBV/MP (4.5%), HIV/HBV (4.0%), HIV/MP (3.1%), TB/MP (2.7%), HBV/HCV (2.2%), HCV/MP (0.4%), TB/HCV (0.4%), HCV/syphilis (0.4%), and TB/HBV (0.4%). On univariate analysis, there was no significant difference ($p>0.05$) in the prevalence of all the co-infections in the patients with respect to gender (Table 1).

The age-related prevalence of co-morbidities were 0.9%, 3.6%, 34.1%, 39.0%, 16.6%, 4.9% and 0.9% among age groups <10, 11-20, 21-30, 31-40, 41-50, 51-60 and >60 years respectively (Table 2). The frequency of co-morbidities was highest in the age groups 21-30 years (34.1%), 31-40 years (39.0%) and 40-50 years (16.6%). Although the most frequent co-morbid infection (i. e. HIV/TB) among the patients also occurred most frequently in these three age groups (82.0%, 164/200), this was not significantly different from the frequency of HIV/TB co-infection in other age groups (78.3%, 18/23) (OR=1.265, 95% CI=0.440-7.633, p=0.5833) (Table 2).

Table 1: Univariate analysis of co-infections among patients attending Kogi State University Teaching Hospital (2012-2021) in relation to gender

Co-infection	No of patients	No of male (%)	No of female (%)	OR	95% CI	p value
HIV/TB	182	86 (47.3)	96 (52.7)	1.400	0.7008 - 2.796	0.3879
HIV/MP	7	1 (14.3)	6 (85.7)	0.1898	0.02245 - 1.604	0.1287
HIV/HBV	9	2 (22.2)	7 (77.8)	0.3257	0.06611 - 1.605	0.1853
HBV/MP	10	6 (60.0)	4 (40.0)	1.828	0.5012 - 6.667	0.5184
TB/MP	6	3 (50.0)	3 (50.0)	1.192	0.2352 – 6.040	1.000
HCV/MP	1	0	1 (100.0)	0.3919	0.01578 – 9.732	1.000
TB/HCV	1	1 (100.0)	0	3.591	0.1446 – 89.184	0.4574
HCV/syphilis	1	0	1 (100.0)	0.3919	0.01578 – 9.732	1.000
HBV/HCV	5	2 (40.0)	3 (60.0)	0.7867	0.1288 – 4.804	1.000
TB/HBV	1	1 (100.0)	0	3.591	0.1446 – 89.184	1.000
Total	**223**	**102 (45.7)**	**121 (52.3)**			

HIV= Human immunodeficiency virus, TB= Tuberculosis, MP= Malaria Parasite, HBV= Hepatitis B virus, HCV= Hepatitis C Virus; OR=Odds Ratio; CI = Confidence Interval, p<0.05 is considered statistically significant

Table 2: Age group distribution of patients with co-morbidity attending Kogi State University Teaching Hospital (2012-2021)

Co-infections	Age group (years)							
	≤10	11 - 20	21 – 30	31 – 40	41 – 50	51 - 60	≥60	Total
HIV/TB	2 (1.1)	6 (3.3)	60 (32.9)[+]	75 (41.2)[+]	29 (16.0)[+]	9 (5.0)	1 (0.5)	182
HIV/MP	0	0	3 (42.9)	1 (14.3)	2 (28.6)	1 (14.3)	0	7
HIV/HBV	0	2 (22.2)	4 (44.4)	2 (22.2)	0	1 (11.1)	0	9
HBV/MP	0	0	2 (20.0)	5 (50.0)	3 (30.0)	0	0	10
TB/MP	0	0	1 (16.7)	4 (66.7)	1 (16.7)	0	0	6
HCV/MP	0	0	1 (100.0)	0	0	0	0	1
TB/HCV	0	0	0	0	0	0	1 (100.0)	1
HCV/syphilis	0	0	1 (100.0)	0	0	0	0	1
HBV/HCV	0	0	4 (80.0)	0	1 (20.0)	0	0	5
TB/HBV	0	0	0	0	1 (100.0)	0	0	1
Total	**2 (0.9)**	**8 (3.6)**	**76 (34.1)**	**87 (39.0)**	**37 (16.6)**	**11 (4.9)**	**2 (0.9)**	**223 (100)**

HIV= Human immunodeficiency virus, TB= Tuberculosis, MP= Malaria Parasite, HBV= Hepatitis B virus, HCV= Hepatitis C Virus; [+] = The combined frequency of HIV/TB co-infection of the three most commonly affected age groups (82.0%, 164/200) was compared with the frequency of HIV/TB co-infection in other age groups (78.3%, 18/23) by Chi square test (OR=1.265, 95% CI=0.4407.633, p=0.5833, not significant).

Discussion:

The global economic burden of co-existence of infectious diseases varies differently from one part of the world to another with regional and population specific patterns. In this study, the frequency of HIV/TB co-infection among the 223 patients with co-morbidity in our study was 81.6%. This indicates that TB among people living with HIV is an emergency in this part of the world where health systems are averagely weak with greater burden of co-morbid infectious diseases. This result affirmed the previous report of WHO that sub-Saharan Africa bears 80% of global burden of HIV-associated TB (9). Therefore, there is need for a well-coordinated approach toward control, diagnosis, and concurrent treatment and management of HIV-associated TB in this region.

The HBV/MP (4.5%), HIV/MP (3.1%), TB/MP (2.7%) and HCV/MP (0.4%) pattern of malaria co-morbid relationship with other infectious diseases obtained from this study indicates that people living with HBV, HCV, HIV and TB in malaria-endemic regions are at the risk of malaria and its complications. The use of insecticide-treated nets (ITNs), indoor insecticide spraying of vector mosquitoes and intermittent preventive treatment (IPT) should be encouraged among these high-risk groups of patients. The pharmacokinetic interactions between malaria drugs and certain antiretroviral (ARV) and anti-TB drugs have been reported to share toxicity profiles (12,13). For this reason, treatment of viral hepatitis or TB and malaria co-infections in an individual should be closely monitored to avoid adverse drug reactions.

A co-morbid interaction between HIV/

HBV of 11% was reported in Nasarawa (14), 9.7% in Niger Delta (15) and 9.2% in Lagos (16). Similarly, HBV/HCV co-morbid prevalence of 5-20% was reported by WHO (7), 5% in Nasarawa (14) and 0.5% in Maiduguri (17). A 4% and 2.2% prevalence of co-infections for HIV/HBV and HBV/HCV respectively was reported in our study, which shows patho-epidemiological pattern of HIV/HBV and HBV/HCV co-morbid in individuals accessing our tertiary healthcare facility. The variations between our study and previous reports may be related to the differences in environmental factors and sociocultural practices or probably related to uncommon practice of intravenous drug abuse in our environment. The frequency of co-morbidities of 0.4% each for HCV/MP, TB/HCV, HCV/syphilis, and TB/HBV in our study appear insignificant but this may be a cause for concern especially in the area of control, diagnosis, and concurrent treatment and management of these co-morbid infectious diseases in an individual.

Co-existence of more than one acute or chronic infectious disease in a person either concurrently or sequentially due to their mode of transmission and common risk factors can increase morbidity and mortality risks when compared to a person infected with only one of these infectious diseases.

Conclusion:

Co-morbid patho-epidemiological patterns of six infectious diseases obtained from our study population is concerning for patients, healthcare providers and global communities. Therefore, diagnosis and concurrent treatment and management of co-morbid infectious diseases should be incorporated into routine health system and appropriate resources should be allotted for this in health plans.

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Conflict of interest:

No conflict of interest is declared

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