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EBOLA VIRUS DISEAES (EVD)

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ABSTRACT

Since the first reported outbreak of Ebola in 1976, there have been approximately 25 outbreaks all of which, except two, have been reported only in east and central Africa. The current outbreak and a single case reported in 1994 in Ivory Coast are the only two outbreaks in West Africa (7). However, the current outbreak, which stared in Guinea (Bissau) in March 2014, remains the deadliest today and the epidemic is still ongoing. New cases are reported daily, particularly in Liberia. This outbreak is unprecedented in many ways. It is the most persisting, lasting more than five months. The spread is across nations and has the largest number of victims. Close to 1500 individuals are dead and very close to 3000 people are infected. More doctors and nurses and other health care workers are infected when compared with previous outbreaks. Over 240 healthcare workers are infected with more than 120 deaths (7). This outbreak also has the least fatality when compared to previous outbreaks. So far, 47% of those infected survive the disease. This work outlines the previous outbreaks and gives a brief summary of current knowledge about EVD.

INFECTIONS DE VIRUS D'EBOLA (VEB)

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RESUME

Depuis le premier cas d'épidémie rapporté en 1976, il y a eu environ 25 foyers épidémiques tous, excepté deux, ont été signalés seulement en Afrique orientale et centrale. L'épidémie actuelle et un seul cas rapporté en 1994 en Côte d'Ivoire sont les deux seuls foyers épidémiques d'Afrique de l'Ouest.Cependant, l'épidémie actuelle reste la plus meurtrière à ce jour et l'épidémie est toujours en cours. Des nouveaux cas sont signalés chaque jour, particulièrement au Libéria. Cette épidémie est sans précédent à bien des égards. Elle est la plus persistante, durant plus de cinq mois. La propagation est entre les nations et a le plus grand nombre de victimes. Près de 1500 personnes sont mortes et près de 3000 personnes infectées. Plusieurs médecins et infirmiers et autres travailleurs de santé sont infectés par rapport aux épidémiesprécédentes. Plus de 240 travailleurs de santé sont infectés avec plus de 120 décès. Cette épidémie a également le moins de décès par rapport aux épidémies précédentes. Jusqu'à présent, 47% de personnes infectées survivent à la maladie. Ce travail présente les épidémies précédentes et donne un bref résumé des connaissances actuelles sur les infections de virus d'Ebola.

INTRODUCTION

Ebola virus disease is caused by Ebola Virus which was identified in 1975 near the Ebola river valley in Zaire during an outbreak in that country. Since 1976, frequent outbreaks have been reported in parts of central and East Africa. Ebola virus is an aggressive pathogen that causes hemorrhagic fever considered one of the most lethal. The mortality rate ranges from 50 to 90%. Outbreaks have been confined to central and East Africa until March of this year when cases were initially reported in Guinea, then Sierra Leone, Liberia and just recently in Nigeria. This is the first time Ebola virus disease outbreaks have been reported outside East and Central Africa. This West African outbreak is the largest outbreak in history and also the most persistent killing more than 1000 people as of today. Also, the number of cases from the current outbreak, which is ongoing, exceeded the number from all previous outbreaks put together. The most worrisome, is the reported cases in Lagos because of the huge population of that city. Lagos population is almost as large of those of Guinea, Sierra Leone and Liberia all together.

EPIDEMIOLOGY

All the outbreaks since 1976 were in central and east

Africa except the lone death in Cote d'Ivoire in 1994. I don't have the details of that single case in Cote d'Ivoire (Table 1). It may be related to an outbreak in that happened about the same time in Gabon. And indeed, that single case may represent the origin of current West African outbreak. We may never know. Out of the 25 reported outbreaks since 1976, only the current epidemics probably originated from West Africa. To date, this outbreak represents the most lethal involving four countries across West Africa.

TABLE 1: CHRONOLOGY OF PREVIOUS EBOLA VIRUS DISEASE OUTBREAKS

Year	Country	Ebolavirus species	Cases	Deaths	Case fatality
2012	Democratic Republic of Congo	Bundibugyo	57	29	51%
2012	Uganda	Sudan	7	4	57%
2012	Uganda	Sudan	24	17	71%
2011	Uganda	Sudan	1	1	100%
2008	Democratic Republic of Congo	Zaire	32	14	44%
2007	Uganda	Bundibugyo	149	37	25%
2007	Democratic Republic of Congo	Zaire	264	187	71%
2005	Congo	Zaire	12	10	83%
2004	Sudan	Sudan	17	7	41%
2003 (Nov-Dec)	Congo	Zaire	35	29	83%
2003 (Jan-Apr)	Congo	Zaire	143	128	90%
2001-2002	Congo	Zaire	59	44	75%
2001-2002	Gabon	Zaire	65	53	82%
2000	Uganda	Sudan	425	224	53%
1996	South Africa (ex-Gabon)	Zaire	1	1	100%
1996 (Jul-Dec)	Gabon	Zaire	60	45	75%
1996 (Jan-Apr)	Gabon	Zaire	31	21	68%
1995	Democratic Republic of Congo	Zaire	315	254	81%
1994	Cote d'Ivoire	Taï Forest	1	0	0%
1994	Gabon	Zaire	52	31	60%
1979	Sudan	Sudan	34	22	65%
1977	Democratic Republic of Congo	Zaire	1	1	100%
1976	Sudan	Sudan	284	151	53%
1976	Democratic Republic of	Zaire	318	280	88%

Figure 1. Obtained from WHO Fact sheet on EVD at www.who.org. Retrieved Aug, 25th , 2014.

VIROLOGY

Ebola Virus is closely related to the Marburg virus, and they both belong to ***Filovideae*** family. The familyname came from their characteristics thread-like appearance. (*filo* is Latin word for filament) This unique morphology provides easy identification on clinical samples using electron microscope. Filoviruses are elongated structure of about 80 nm in diameter. The length of the replicate form is about 970 for Ebola. Ebola nucleocapside is about 50nm and helical in nature and surrounded by a membrane consisting of many projecting spikes. The genome consists of seven open reading frames, which encode structural proteins such as the virion envelope protein or GP, nucleoprotein or NP, matrix proteins VP24 and VP40. There are other none structural proteins such as the VP30 and VP35(3). It also contains other proteins such as the polymerase enzyme.All filoviruses have multiple copies of a single membrane attached glycoprotein(GP), which project from the viral envelope (1,3 4). But unlike the other filoviruses such as the Marburg, Ebola GP open reading frame of Ebola has two gene products; a soluble 60- to 70 kDA protein called sPG and another full length 150- to 170 kDA protein which is directly inserted into the viral memebrane(2). The Ebola virus GP is a good target for multiple neutralizing antibodies (1). In fact, a few have been tested in none-humans and found to be effective in inducing the production of neutralizing antibodies (1).


Photomicrograph of Ebola virus
credits: streamafrica.com

CLASSIFICATION

Ebola and Marburg viruses are members of the ***Filoviridae*** family and are pleomorphic negative-sense RNA viruses. Their genomic structure is closely related to those of ***Paramyxoviridae***. There are five identified strains of Ebola virus of which three are known human pathogens. They are, Zaire, Ivory Coast (Bundibugyo) and Sudan strains. Sudan strain is considered the most lethal (3). The fourth and the fifth subtypes called the Reston and Tai forest respectively are yet to be associated with epidemics. Reston subtype was discovered in Reston Virginia in the United States in 1989-1991 from dying cynomolgus monkeys imported from the Philippines. There are episodes of human infections with this strain but with no medical consequences

PATHOGENESIS

The exact manner in which Ebola virus produces EVD is yet to be fully understood. However, it is known that viraemia persists throughout the acute period. When viraemia disappears, patients are normally well and antibodies appear in the patient blood (7). Therefore, it is assumed that the effective immunity response is not humoral. Monocytes, macrophages, and dendritic cells are the early targets of the virus (4). The destruction of these cells play a central role because proinflammatory and antiviral cytokins such as IFN-α, Interlukins, IL, 1,6,8, and 12, tumor necrosis factor, TNF family members and coagulations factors 11, and 13-18 blood levels are markedly increased (4). Extensive viral replication is seen in most of the major organs as well as in cells of the endothelia, epithelia and monocytes lineage of infected individuals and primates. There is severe dysregulation of the vascular and inflammatory response, which play a key role in EVD manifestations.

It has been demonstrated that EBV suppresses host antiviral response, including the Toll-like receptor, (TLR). Interferon (IFN), regulatory factor-3, and protein kinase R (PKR)- mediated pathway in human hepatocytes (4). Also, it was found that when EBV particles attaches itself and enter human macrophages, that resulted in induction of proinflammatory mediators such as IL-6, IL-8 and tumor necrotic factor alpha (TNF-α) (4)

The EBV GP is fingered in some of the cell destructions and the pathogenesis. GP appear to bind preferentially to endothelial cells. The exact receptors for cell binding and infection are not understood (4). There are two types of GP, secreted and transmembrane or sGP. GP allows the virus to introduce its contents to monocytes and macrophages, which leads to cell damage and the release of cytokins. This in turn leads to inflammation and fever. GP also allows the attachment to endothelial cells causing the vascular damages and the subsequent bleedings. Secretary GP or sGP is thought to inhibit neutrophils which would have assisted in viral clearance (4).

IMMUNOLOGY

Immune response to EBV is initially poor because the virus replication is so fast such that the protein synthesis of the infected cell is overwhelmed. The exact component of the immune system that protects against EBV infection is yet to be defined. Protection from serum of convalescent patients has not been found to be consistent. Besides, serum from survivals has not consistently altered the progress of the disease in clinical practice (4). However, a recent study (4) suggests that mononuclear antibody from bone marrow of recovered patients has been shown to confer immunity in murine model of Ebola infection (4).

Cell mediated immunity is thought to play a significant role, but the method is yet to be elucidated. Cytotoxic T lymphocytes are known to provide protection against intracellular organism such as EBV, but their role in EBVD is not well defined. Wilson et al (2000) vaccinated mice with Venezuelan equine encephalopathy virus replicons encoded with EBV NP and then injected the mice with a lethal dose of EBV. The mice survived and they noticed induced antibodies to EBV NP and Major Histocompatibility Complex class-1 restricted CTL (4). This has not been repeated in any other animals but it may provide future clues about the role of CTL in EBOV protection.

TRANSMISSION

Ebola virus disease is a zoonotic diseases and introduction into human population is generally through human contact with blood, feces, secretions, organs or body fluids of infected animals. Consumption of infected bush meats particularly those of apes are thought to be one of the main contact methods. Handling of infected chimpanzees, monkeys, gorillas, antelopes, porcupines and fruits bats is considered the major epidemic sources in Africa.

After the initial infection from animal source, community spread is from human-to-human contacts. Body fluids, blood and indirect contact with the environmental objects that are previously contaminated with infected fluids. Sometimes transmission may be at burial ceremonies where the living have direct contacts with the dead. Recovered men are capable of transmitting the virus up to six months after recovery (4,7).

Health care workers are at a greater risk of acquiring the disease while treating infected individuals. In fact, *I just heard the news from CNN at this moment that the current epidemics has affected 240 health care workers of which about 120 healthcare personnel are dead (CNN, international News Report, 26/8/2014).

CLINICAL PRESENTATION

EVD is an acute severe illness with sudden onset characterized by fever, intense fatigue, myalgia, headache and sore throat (1, 2, 4, 5.8, 9). Incubation period is from two to 21 days. There may be nausea and vomiting with abdominal cramps mimicking several other tropical illnesses. Diarrhea, cough and chest pain may complicate the diagnostic approach. Other common features are pharyngitis, photophobia, internal and external bleeding from the mouth, ears and nose. There may be blood in sputum and urine. CNS involvement, lymphadenophathy, jaundice and pancreatitis are not uncommon. A prominent maculopapular rash around the trunk is commonly seen around the 5th day. Wasting and bleeding manifestations are also common about the same time. By the second week, patient either begins to show marked improvement or die from multiorgan dysfunctions (9). Survivors may be left with orchitis, recurrent hepatitis, transverse myelitis or uveitis (9) Mortality from EVD may be as high as 90% but in general, Zaire subtype is the most lethal.

DIAGNOSIS

The following diseases must be excluded before EVD is considered, particularly in a tropical setting; malaria, typhoid fever, shigellosis, cholera, leptospirosis, plague, rickettsiosis, relapsing fever, meningitis, hepatitis, and other viral hemorrhagic fevers like yellow fever and Marburg virus disease (7). During epidemics, healthcare workers must practice universal precautions when attending to all patients.

Laboratory diagnosis rests on identification of the virus, its genetic markers or the viral antigen. Real-time PCR is one of the strong diagnostic tools today because of speed and specificity. Cell culture, when possible may be considered. Its classified as a level 4 pathogen, so only few laboratories are qualified to culture the virus. Other rapid tests that can easily be performed with proper precautions include, antibody-captured enzyme-linked immunoabsorbent assay (ELISA), antigen detection tests (antigen-catch ELISA), serum neutralization test.

The patient's blood or serum is extremely infective, so care and precautions must be adequate. Because seroconvertion is between day 8 and 12, antigen detection should be the test of choice during the acute stage. IgM detection is appropriate during the convalescent period. IgG does not play any role in the diagnosis of EVD (9)

Preliminary tests such as a full blood count will indicate thrombocytopenia and leucopenia. Liver function test will also show elevated transaminases, and particularly when aspartate transaminases are more elevated than alanine transaminases, and when combined with the stated full blood count anomalies above, then filovirus infection should be suspected (4, 7, 9).

TREATMENT

There are few drugs and vaccines under development and, in fact, some are awaiting clinical trials. There is no specific treatment available yet for EVD; and no vaccine is licensed yet for human use.Treatment is basically those of intensive supportive care and proper hydration. Attention must be focused on correcting electrolyte imbalance. Replacement of coagulation factors and platelets may be necessary.

When clinical or laboratory evidence suggests intravascular coagulation, then heparin or other treatment forms for DIC may be necessary (7).

PREVENTION

African fruit bats of three genera are considered the natural habitat of Ebola virus. Fruit bats of the genera *Hypsignathus monstosus, Epomops franqueti, and Myonycte ristorquata* are thought to be the natural hosts for the virus. However, primates have been the main source of the infection to man. Several outbreaks have been seen in Gorillas and Chimpanzees (7). Therefore, reducing human contacts, particularly consumption of these animals is imperative for Africans to control animal-to-man transmission, which is often the primary source of epidemics. It is extremely important for people not to touch or play around such animals that are found dead or sick without a known cause.

During outbreaks, public education to raise awareness and increase knowledge should be the primary focus of public health practitioners and the government. Protective measures that individuals can take must be emphasized and information should be disseminated very efficiently and rapidly. In Africa, where outbreaks are common, the goal of public education and awareness should include the following; reducing the risks of animal-to-human transmission, reducing the risk of human-to-human transmission, and community affected need to be informed on methods to abort the spread of the disease.

Healthcare workers should always use standard precautions since they do not know when a new case will arise. There is no season for Ebola outbreaks, so standard precautions should be practiced at all times. Those caring for patients with suspected or confirmed cases should use ultimate infection control measures to ensure complete absence of exposure to blood or body fluids. Sterile gloves, face masks, long sleeve coats, protective shoes and goggles should be available to those who make close contacts with the patient. Trained laboratory workers in suitable (category 4 biohazard) laboratories should handle clinical specimens (7)

The role of platelets and cytotoxic T-lymphocytes from patients who have recovered from the disease in the passive immunization of patients and people who have been exposed to patients should be further explored. Lastly, the value of pooled serum of survivors in patients' management should also be explored further.

REFERENCES

1. Tran, E. E., Simmons, J. A., Bartesaghi, A., Shoemaker, C. J., & Nelson, E. (2014) Spatial localization of the Ebola virus mucin-like domain determined by cryo-electron tomography. *J. of virology, 88(18): 1098: DOI: 10.1128/JVI.00870-14*
2. Gupta, M., Mahanty, S., Greer, P., Towner, J. S., &Shiel, W. (2004) Persistent infection with Ebola virus under condition of partial immunity. *J of Virology, 78(2):958. DOI:10.1128/JVI.78.2.958-967.2004*
3. Sullivan, N., Yang, Z., &Nabel, G. J. (2003) Ebola virus pathogenesis: Implication for vaccines and therapies. *J. of virology, 77(18):9733.DOI:10.1128.JVI.77.18.9733-9737.2003.*
4. Kindrachuk, J., Wahl-Jensen, V., Sanfronetz, D., Trost, B., &Hoenen, T. (2014) Ebola virus modulates transforming growth factor β signaling and cellular markers of mesenchyme-like transition in hepatocytes. *J. of virology, 88(17):9877.DOI:10.1128/JVI.01410-14.*
5. Regnery, R. L., Johnson, K. M., Killey, M. P. (1980) Virion nucleic acid of Ebola virus. *j. of virology, 36(2): 465.*
6. Han, Z., Lu, J., Liu, Y., Davis, B., & Lee, M. S. (2014) Small-molecule probes targeting the viral PPxY-host NEdd4 interface block egress of a broad range of RNA viruses. *J. of Virology, 88(13):7294. DOI: 10.1128/JVI.00591-14-*
7. WHO (2014) Fact sheet on Ebola Virus Diseases. Published by the WHO and Retrieved from www.who.org . 25th August, 2014.
8. WilsoN, J. A., & Hart, M. K. (2001) Protection from Ebola virus mediated by cytotoxic T lymphocytes specific for the viral nucleoprotein. *J. of virology, 75(6):2660.DOI:10.1128.JVI.75.6.660-664.2001*
9. Mandell, G. L., Bennett, J. E., & Dolin R. (2005) *Principle and Practice of Infectious Diseases.* 6th Edi. Elsevier Churchill Livingstone. Philadelphia, PA.
10. Watt, A., Moukambi, F., Banadyga, L., Groseth, A., &Callison, J. (2014) A novel life cycle modelling system for Ebola virus shows a genome length-dependent role of VP24 in virus infectivity. *J. of virology, 88(18):10511.DOI:10.1128/JVI.01272-14.*

ORIGINAL ARTICLE

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POLYMERASE CHAIN REACTION (PCR) PROVIDES A SUPERIOR TOOL FOR THE DIAGNOSIS OF PNEUMOCOCCAL INFECTION IN BURKINA FASO

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ABSTRACT
Purpose of study: The aim of this study was to determine the value of real-time Polymerase Chain Reaction (rt-PCR) in the routine surveillance of pneumococcal meningitis in Burkina Faso, compared to standard methods of culture, Gram stain and latex agglutination assay.
Materiel and methods: A total of 385 specimens of cerebrospinal fluid were analyzed by the three standard bacteriological methods (Gram stain, latex agglutination assay, and culture) and real-time Polymerase Chain Reaction.
Results: Of 385 specimens analyzed by these methods, 204 *S. pneumoniae* were detected by one or more methods. Gram stain detected 36.4% (140/385) Gram positive encapsulated diplococci; 37.7% (145/385) and 20.8% (80/385) of the specimens were positive for pneumococci by latex agglutination assay and culture. These specimens were tested with rt-PCR, which confirmed 51.2% (197/385) *S. pneumoniae* positive. The sensitivity and specificity of culture were 54.4% and 31.5%, respectively, and the sensitivity and specificity of rt-PCR were 96.6% and 100%, respectively. These results showed that rt-PCR was more sensitive than Gram stain (*p=0.0235*), latex agglutination assay (*p=0.0442*)and culture (*p=0.0006*).The culture is the gold standard method; however, the result showed that rt-PCR had specificity and was as specific as Gram stain (*p=0.3405*) and latex agglutination assay (*p=0.7745*).
Conclusion: rt-PCR was highly sensitive and specific. It could be used as a complementary diagnostic tool to improve case confirmation of bacterial meningitis. However,its high cost, the qualification of the technical staff and infrastructures required for its implementation, constitute obstacles to its widened use in countries with limited resources.
Keywords: *Streptococcus pneumoniae*, meningitis, rt-PCR, standard bacteriological methods

REACTION DE POLYMERISATION EN CHAINE, UN OUTIL SUPERIEUR POUR LE DIAGNOSTIC DES INFECTIONS PNEUMOCOCCIQUES AU BURKINA FASO

Chaibou, Y.[1,2*], Congo/Ouedraogo, M.[1], Sanou, I.[1,3], Somlaré, H.[1], Ouattara, K.[1], Kienou, C.M.[1], Belem, H.[1], Sampo, E.[1], Traoré, S.A.[2], Traoré/Ouedraogo, R.[3,4], Hatcher, C.[5], Mayer, L.[5], Wang, X.[5], Sangaré, L[1,3].

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RESUME
Objectif: Le but de cette étude était de déterminer la place de la rt-PCR dans la surveillance de routine de méningitespneumococciquesau Burkina Faso et la comparée avec les méthodes de la bactériologie classique: Culture, coloration de Gram et l'agglutination au latex.
Matériel etméthodes: Au total, 385 échantillons deliquides céphalorachidiens (LCR)étaient analysés par les trois méthodes de la bactériologie classique (coloration de Gram, agglutination au latex, culture) et la PCR en temps réel.

RESULTATS: **Parmi 385 échantillons analysés, 204 cas de *Streptococcuspneumoniae* étaient détectés par une ou plusieurs méthodes. La coloration de Gram adétecté 36,4% (140/385) diplocoques encapsulés à Gram positif (DGP); 37,7% (145/385) et 20,8% (80/385) d'échantillons étaient positifs aux pneumocoques par l'agglutination au latex et la culture. Ces échantillons étaient aussi testés par rt-PCR qui a confirmé 51,2% (197/385) cas positifs de *S. pneumoniae*. La sensibilité et la spécificité de la culture étaient respectivement de 54,4% et 31,5%, et la sensibilité et la spécificité de rt-PCR étaient respectivement de 96,6% et 100%. Ces résultats ont montré que la rt-PCR était plus sensible que la coloration de Gram (*p=0,0235*), l'agglutination au latex (*p=0,0442)* et la culture(*p=0,0006*).La culture est une méthode de référence; cependant, le résultat a montré que rt-PCR était plus sensibleet aussi spécifique que la coloration de Gram ((*p=0,3405*)et l'agglutination au latex (*p=0,7745*).**

Conclusion : :rt-PCR était plus sensible et plus spécifique. Elle pourrait être utilisée comme un outil de diagnostic complémentaire pour améliorer les cas de confirmation de méningites bactériennes. Cependant, ces coûts de réalisation, la qualification de techniciens et les matériels pour son application constituent des obstacles pour sa vulgarisation dans les pays à ressources limités.

Keywords: *Streptococcus pneumoniae*, méningites, rt-PCR, méthodes de la bactériologie classique

INTRODUCTION

Streptococcus pneumonia (pneumococcus) is a common pathogen associated with benign or severe infections including acute otitis media, meningitis, septicemia and pneumonia (1). According to WHO annual reports, 1.6 million cases of fatal pneumococcal disease occurredworldwide annually, mostly in infants and elderly(2, 3). However, many countries have implemented pneumococcal conjugate vaccine during the last few years and number of cases has declined significantly (4-7). *S. pneumonia* is fastidious and sensitive to temperature variation and thereforerequires rapid and accurate identification for proper and timely antibacterial therapyand epidemiologic surveillance. Bacterial etiology is typically identified and characterized usingstandard methods such as Gram stain, latex agglutination assay, culture, susceptibility to optochin (ethyl hydrocupreine hydrochloride)and bile solubility(8-10).The probability to detect pneumococcus in CSF by Gram stains and culture is determined by the bacterial concentration in a CSF specimen (8). Gram stain is a simple, rapid and inexpensive method but the probability to detect pneumococcus in CSF by this methodwas determined by the bacterial concentration in a CSF specimen (8). Although, the culture is cheap, it is difficult to perform on every specimen collected. In many developing countries, surveillance for bacterial meningitis is hampered by limited use of culture and a high frequency of negative cultures (11). Toovercome the limitations of the standard bacteriological methods, molecular methodswere introduced in many countries to improve the detection of etiologic agents. In Africa, particularly in Burkina Faso, multiplex PCR was implemented to identify *Neisseria meningitidis*, *Haemophilusinfluenza*e serotype b and *S. pneumonia* simultaneously in an effort to evaluate the usefulness of PCR technology in meningitis surveillance(12). In another study, PCRidentified 27% pneumococci fromCSF specimens but cultureand latex agglutination assayonly detected 9% and 15%, respectively (13): the results reported in these studies showed that PCR were more sensitive than culture and latex agglutination assay. The aim of this study was to determine the value of rt-PCR in the routine surveillance of pneumococcal meningitis in Burkina Faso, compared to standard methods of culture, Gram stain and latex agglutination assay.

MATERIAL AND METHODS

Study sites

The study was conducted in the Bacteriology and Virology Departmentof the Teaching Hospital YalgadoOuedraogo (TH-YO)in Ouagadougou, Burkina Faso. The specimens were collectedfrom ninemedicalregions of Burkina Faso: Center, Centre-West, Center-South, Center-North, Central Plateau, East, North, Boucle of Mouhoun and the Sahel, from March 2010 toDecember 2012.

Clinical specimen collection

The cerebrospinal fluid (CSF) was obtained from patients with suspect meningitis. A case of suspect meningitis was defined by rapid onset of fever (>38.5°C rectal or >38.0°C axillary) followed by stiff neck, headache, altered consciousness, bulging fontaneland convulsion for infant, vomiting and coma, and a confirmed meningitis that was laboratory-confirmed by growing (culturing) or identifying (by Gram stain, antigen detection, or PCR) a bacterial pathogen (*S. pneumoniae*) in CSF of patients with a clinical syndrome consistent with bacterial meningitis (14). Lumbar puncture was performed for the cases of suspect meningitis and three or five milliliters (3-5mL) of CSF specimen werecollected and transferred into asterile tube for laboratory confirmation. The specimens collected at the teaching hospitalof Ouagadougou weretransported to the laboratory in less than one hourafter collection.

For the specimens coming from other medical regions, 1 mL of CSFwas inoculated intoTrans-Isolate (T-I) medium for cultureand 0.5mL of CSF into cryotubes for PCR in less than one hour after collection. All cryotubes were stored at -80°C until their analyses.

Analyses of CSF specimens

Gram stain

Gram stain was conducted either in the department of bacteriology and virology laboratory on CSFs collected at the teaching hospital YalgadoOuedraogo or in the local laboratories on CSFs collected in other medical regions. The Gram stain results were reported on the notification forms before transferring the CSFs and forms to the bacteriology and virology department in the teaching hospital.

A CSF was considered positive for *S. pneumonia* if Gram positive diplococcic and/or cocci encapsulated in short chains were observed during the microscopic examination with a clear halo around the bacterial cells. A Gram stain was considered negative if no bacteria or any other types of bacterial cell morphology wereobserved.

Latex agglutination assay

The PASTOREXTMMeningitis kit (Bio-Rad, France) was used country wide in Burkina Faso for the direct detection of the capsular antigen of *S. pneumoniae.* The test was performed according to the manufacturer's instruction.

Bacterial isolation and identification

To isolate *S. pneumoniae*, 1or 2 drops of CSF specimenor inoculated T-I medium wasplatedontoa chocolate agar plate and/or ontoa blood agar plate (Trypticase-soya agar supplemented with 5% sheep blood). All plates were incubated overnight at 37°C, with 5% CO2.

Colonies with typical pneumococcal morphology (moist or sometimes mucoid, gray, central depression) were tested for catalase andα-hemolysis on blood agar plate or egg yolk-like hemolysis on chocolate agar plate. Susceptibility to optochin (5µg; Bio-Merieux, France)was performed on the catalase-negative and α-hemolytic colonies for the presumptive identification. Any isolates that produced an inhibition zone with diameter equalor largerthan 14mmwere considered susceptible.Isolates with smaller inhibition zone or without inhibition zone were tested with the bile solubility test using 2% sodium deoxycholate.

An isolate was identified as *S. pneumonia* if it was Gram-positive for pneumococci, producedα-hemolysis on blood agar plate or egg yolk-like hemolysis on chocolate agar plate, was catalase negative and optochin-susceptible or solubilized by bile salt solution when resistant to optochin. All *S. pneumonia* strains isolated were stored at -80°C in 10% Skim milk glycerol.

Real-time PCR (rt-PCR)

-DNA Extraction:100uL of saline containing 0.04g/mL of lysozyme (Sigma-L-6876) and75U/mlof mutanolysin (Sigma-M9901) was pipetted into themicrocentrifuge tube.Then, 200µl of CSF was added into the microcentrifuge tube. DNA extraction was performed using QIAamp® DNA Mini Kit (Qiagen S.A., France) according to the manufacturer instructions. Extracted DNA samples were stored at -20°C.

-DNA amplification: The *lytA*rt-PCR, targeting the autolysin gene*lytA*, was performed as described previously (15), with the following modifications. The assays were carried out in a final 25µl reaction volume and were performed using12.5µl of Master Mix TaqMan® Universal PCR (Applied Biosystems), with 2µl of sample extracted DNA. Forward primer, reverse primer, and probe for each target gene were used in concentrations of 200 nM(15). The PCR cycling conditions were 1 cycle of 50°C for 2 minutes, 1 cycle of 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 60°C for 1 minute. The thermal cycler Stratagene Mx3005PTM (Agilent La Jolla Technologies, California, USA) was used for PCR amplification. The results were analyzed by MxPro (Mx3000P/Mx3005P) software.

Interpretation of PCR results: A positive result was defined as a cycle threshold (Ct) value was ≤35 cycles; and in conclusive or equivocal result as the Ct was between >35cyclesand <40 cycles, and the negative result as a Ct value was ≥40 cycles. All inconclusive or equivocal results were diluted to 1:4 and 1:10and PCR were repeated on the dilutions. In order to assess the performance of rt-PCR in detection of *S. pneumoniae*, we considered the culture as the gold standard method to determine the sensitivity, the specificity, positive predictive value (PPV) and negative predictive value (NPV) of rt-PCR.Specimens that were culture positive for another organism were excluded.

Ethical aspects: All specimens were collected as part of the routine clinical management of patients, according to the national guidelines in Burkina Faso. The study was approved by the medical establishment committee of Teaching Hospital YalgadoOuedraogo.

Statistical analyses

Epi-Info version 3.5.1 and MedCal 11.0.1.0 were used to compare the performance of rt-PCR to those of standard bacteriological methods. The difference was statistically significant when the *p*-value was lower than .05 *($p<0.05$)*.

RESULTS

A total of 385CSFs specimens were collected from suspect casesfor meningitis. *S. pneumoniae* was detected in 204 (51.2%) CSFs specimens,by the three methods (latex agglutination assay, culture and rt-

PCR). As shown in Table 1, among 385 CSFs analyzed by the three confirmatory tests, 140 (36.4%) were Gram positive encapsulated cocci. All these 140 CSFs specimens were positive for *S. pneumonia* by latex agglutination assay, culture or/and rt-PCR. Latex agglutination assay, culture and rt-PCR detected 145 (37.7%), 80(20.8%), and 197 (51.2%)positive cases of *S. pneumoniae*respectively. The results showed (Table 1) that rt-PCR detected more cases than latex agglutination assay *(p=0.0058)* and culture *(p<0.0001)*.Of the 197 *S. pneumoniae* detected by rt-PCR, 57 (14.8%) *S. pneumoniae* were detected from CSFs that were negative by culture and latex agglutination assay (Table 2). Of the 385 CSFs tested by both culture, latex agglutination assay and rt-PCR, 204 (53.0%) were positive by at least one of the three methods (Table 2).

TABLE 1: *S. PNEUMONIAE*CASES DETECTED BY THE STANDARD BACTERIOLOGICAL METHODS AND RT-PCR

Test	Positive (%)	95%CI	Negative (%)	95%CI	*p value*
Gram stain	**140 (36.4)**	**31.2-40.9**	**245 (63.6)**	**56.2-74.9**	**<0.0001**
Latex agglutination	**145 (37.7)**	**32.4-42.2**	**240 (62.3)**	**55.7-74.1**	**<0.0001**
Culture	**80 (20.8)**	**16.6-24.8**	**305 (79.2)**	**69.3-92.6**	***<0.0001***
rt-PCR	**197 (51.2)**	**44.8-54.9**	**188 (48.8)**	**45.1-55.2**	***0,6835***

TABLE 2: CO-DETECTION OF *S. PNEUMONIAE* BY CULTURE, LATEX AGGLUTINATION ASSAY AND RT-PCR

Combination of methods	Numberdetected (%)
rt-PCR+/Culture+/Latex agglutination assay+	**76 (19.7)**
rt-PCR+/Culture–/Latex agglutination assay+	**62 (16.1)**
rt-PCR+/Culture–/Latex agglutination assay–	**57 (14.8)**
rt-PCR–/Culture+/Latex agglutination assay+	**2 (0.5)**
rt-PCR–/Culture+/Latex agglutination assay–	**0**
rt-PCR–/Culture–/ Latex agglutination assay+	**5 (1.3)**
rt-PCR+/Culture+/ Latex agglutination assay–	**2 (0.5)**
Total	**204 (53.0)**

Among the 197 rt-PCR positive specimens, 138 (70.1%) were *S. pneumoniae* positive by latex agglutination assay,and 78 (39.6%) positive by culture. All culture-positive CSF specimens were rt-PCR positive except for two specimens. These specimens were analyzed twice by rt-PCR, and the results remained negative (Table 3). *S. pneumoniae* was detected by culture and/or latex agglutination assay in 7CSFs that were rt-PCR negative.

Sensitivity and specificity of culture, latex agglutination assay and rt-PCR were evaluated using 385 analyzed samples (Table 4A). The rt-PCR assay was more sensitive (96.6%) than Gram stain (67.4%) (*p=0.02*), latex agglutination assay (70.4%) (*p=0.04*) and culture (54.4%) (*p=0.0006*). Their specificity and positive predictive value were 100% (Table 4B). Culture showed a lower specificity (31.5%) compared to latex agglutination assay (95.2%) (*p<0.0001*). These differences were statically significant. There was no significant statistical difference in thenegative predictive values (NPV) (*p=0.8*) between Gram stain (71.8%) and latex agglutination assay (75.0%), and the positive predictive values (83.5% for Gram stain *vs* latex agglutination assay (70.4%) (*p=0.2*).

TABLE 3: COMPARISON BETWEEN RT-PCR AND THE STANDARD BACTERIOLOGICAL METHODS FOR THE DETECTION OF *S. PNEUMONIAE*

Test		rt-PCR		
		Positive (%)	Negative (%)	Total (%)
Gramstain	Positive	126/197 (64.0)	14/188 (7.4)	140/385 (36.4)
	Negative	71/197 (36.0)	174/188 (92.6)	245/385 (63.6)
Latex agglutination assay	Positive	138/197 (70.1)	7/188 (3.7)	145/385 (36.9)
	Negative	59/197 (29.9)	181/188 (96.3)	240/385 (63.1)
Culture	Positive	78/197 (39.6)	2/188 (1.1)	80/385 (20.8)
	Negative	119/197 (60.4)	186/188 (98.9)	305/385 (79.2)

DISCUSSION

Identification of *Streptococcus pneumonia* by culture is essentially based on colony appearance, bacterial cell morphology in Gram stained smears,biochemical characteristics including the optochin susceptibility (ethyl-hydrocupreine hydrochloride) and bile solubility. Molecular tests have been developed in the past several years,to overcome the limitations of standardbacteriologicalmethods (Gram stain, culture and latex agglutinationassay) in order to distinguish pneumococcus (*S. pneumoniae*) from other *Streptococcus* species such as *Streptococcus oralis* and *Streptococcus mitis*.

The advantage of using PCR over culture and Gram stain is that PCRis turnaround time or being able to detect a non-viable organism where a patient may have been on antibiotic treatment at the time of specimen taking. More importantly, PCR is more sensitive than other bacteriological methods. Although culture is still considered as the gold standard (16), its sensitivity can be low due to many factors such as prior antibiotic treatment, the timing of specimen transport, and media quality. According to our results, only20.4% of suspect cases were culture positive; 79.6% samples selected in this study were culture negative.

The low sensitivity of culture entrained a difficulty to identify the infection etiology. In fact, PCR can effectively confirm the results of the standard bacteriological methods and improve the confirmation of pneumococcal disease (17). In our study, rt-PCR showed 96.6% sensitivity and 100% specificity. The positive predictive value was 100%. These results were comparable with those obtained from other studies where the PCR sensitivitywas estimated from 88 to 100% and the specificity from 75 to 100% (18-22). The fact that PCR has shown high specificity and positive predictive value in our study indicates that PCR is unlikely to produce false positive results, and therefore can be used as a reliable tool for pneumococcal meningitis diagnosis. However, a negative PCR result does not indicate the absolute absence of infection as no single test has demonstrated 100% sensitivity; it is possible in thissituation, either the primers were not adequate to the target gene, the probe or the primers were damaged, or there was an inhibitor.

Other PCR platforms such as multiplex PCR were used in other studies to identify the common bacterial meningitis pathogens (12, 19, 20, 23) and reported to produce reliable results with improved turnaround time. The target gene for the detection of *S. pneumonia* used in these PCR assays was the pneumolysin gene (*ply*) (24, 25), which is present in all Streptococcusspecies and not specific to *S. pneumoniae* especially in carriage studies (25). PCR assays targeting genes such as *sod*A (superoxide dismutase A gene) (26), *sp*9802 (fragment 9802 gene) (21), and *psa*A (pneumococcal surface adhesion A gene) (25,27) were less specific than pneumococcal *lyt*APCR (15, 17, 28, 29). Our data showed that rt-PCR targeting autolysin gene *lyt*A was acomplementary method for identification of *S. pneumonia* during the epidemic seasons in Burkina Faso.

TABLE 4: SENSITIVITY AND SPECIFICITY OF DIFFERENT METHODS

A.

		Culture		
		Positive	**Negative**	**Total**
Gram stain	**Positive**	**132**	**26**	**158**
	Negative	**64**	**163**	**227**
	Total	**196**	**189**	**385**
Latex agglutination assay	**Positive**	**140**	**9**	**149**
	Negative	**59**	**177**	**236**
	Total	**204**	**186**	**390**
rt-PCR	**Positive**	**197**	**7**	**204**
	Negative	**0**	**181**	**181**
	Total	**197**	**188**	**385**

B.

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Gramstain	**67.4**	**86.2**	**83.5**	**71.8**
Latex agglutination assay	**70.4**	**95.2**	**70.4**	**75.0**
Culture	**54.4**	**31.5**	**32.9**	**52.8**
rt-PCR	**96.6**	**100**	**100**	**96.3**

PPV: Positive Predictive Value; NPV: Negative Predictive Value

Our results also showed that rt-PCR improved diagnosis of *S. pneumonia* compared to standard bacteriological methods (Gram stain, latex agglutination assay,culture), particularly when thesemethods were negative or inconclusive; however, other tests were still valuable for pneumococcal diagnosis in laboratories where PCR technology was not available. In spite of its low sensitivity (54.4%) and specificity (31.5%), culture remained the gold standard method in the pathogenic diagnosis of this bacteriumand todetermine its sensitivity to antibiotics. Isolates were especially valuable to study the molecular epidemiology of *S. pneumonia* and to identify target genes for developing diagnostic tools. With a sensitivity of 70.4% and a specificity of 95.4%, latex agglutinationassay can be also considered as an alternative in absence of PCR. These results were similar to those of McAvin et *al* (2001) who obtained sensitivity and a specificityof latex agglutinationassayof 96% and 85% respectively (17). These results prove the importance of latex agglutination assayin the diagnosis of pneumococcal meningitis particularly in emergency situations. rt-PCR provides a rapid and reliable diagnostic tool(16). However, this method should not replace the standardbacteriological methods, particularly the culturethat contributesto provide data about the germ susceptibility to antibiotics:there are all complementary in the improvement of the surveillance of pneumococcal infections.

The cost of the rt-PCR constitutes an important limit for its implementation in low-income countries (12). This cost could be minimize by implementing the technique in central laboratory or in national reference laboratory which would receive CSF specimens collected for the confirmations of cases suspected in various sanitary regions of the country.

CONCLUSION

The implementation of rt-PCR in Burkina Fasoallowsconfirmation of more suspect cases of pneumococcal meningitis compared to culture andlatex agglutination assay. Although the standard bacteriological methods remain essential for the diagnoses of pneumococcal meningitis, rt-PCR represents an improvement on the standard bacteriological method's performance because of its sensitivity and specificity. PCR is a rapid and reliable method and can be used as a complementary method for pneumococcal detection. PCR has proven to be a valuable tool in medical laboratories of West Africa countries such as Burkina Faso where culture-based bacterial detection is challenging due to contamination, delay in transport and inappropriate storage of CSF specimens collected in rural medical centers.

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REFERENCES

1. Picard C., et Casanova, J.L.Prédisposition génétique aux infections à pneumocoques. *Rev.Mar. Mal.Enf*.2008; 18:72-76
2. WHO. Pneumococcal conjugate vaccine for childhood immunization-WHO position paper. WER 2007; 82(12): 93-104
3. WHO: 23-valent pneumococcal polysaccharide vaccine WHO position paper. WER 2008; 83(42): 373-384
4. O'Brien, K.L., Wolfson, L.J., Watt, J.P., Henkle, E., Deloria-Knoll, M., McCall, N., Lee, E., Mulholland, K., Levine, O. S., Cherian, T. Burden of disease caused by *Streptococcus pneumoniae*in children younger than 5 years: global estimates.*Lancet*.2009; 374: 893–902
5. Tin TinHtar, M, Madhava, H., Balmer, P., Christopoulou, D., Menegas, D., Bonnet E. A Review of the Impact of Pneumococcal Polysaccharide Conjugate Vaccine (7-valent) on Pneumococcal Meningitis. *Adv.Ther*.2013; 30:748–762.
6. Mackenzie, G.A., Plumb, I.D., Sambou, S., Saha, D., Uchendu, U.,Akinsola, B., Ikumapayi, U.N., Baldeh, I., Usuf, E., Touray, K., Jasseh, M., Howie, S.R.C., Wattiaux, A., Lee, E., Knoll, M.D., Levine, O.S., Greenwood, B.M., Adegbola, R.A., Hill,P.C.Monitoring the introduction of pneumococcal conjugate vaccines into West Africa: design and Implementation of a population-based surveillance system. *PLoS Med*.2012;91): e1001161. doi:10.1371/journal.pmed.1001161
7. Pilishvili, T.,Lexau, C., Farley, M.M., Hadler, J., Harrison, L.H., Bennett, N.M.,Reingold, A., Thomas, A., Schaffner,W., Craig, A.S., Smith, P.J., Beall, B.W., Whitney, C.G., and Moore, M.R. for the Active Bacterial Core Surveillance/Emerging Infections Program Network, Sustained Reductions in Invasive PneumococcalDisease in the Era of Conjugate Vaccine.*J. Infect. Dis*.2010; 201:32–41

8.Gray, L.D., andFedorko, D.P.Laboratory diagnosis of bacterial meningitis. *Clin.Microbiol. Rev*.1992; 5(suppl 2):130

9. Arbique, J.C., Poyart, C., Trieu-Cuot, P., Quesne, G., Carvalho, M-G.S., Steigerwalt, A.G., Morey, R.E., Jackson, D., Davidson. RJ, Facklam, R.R. Accuracy of Phenotypic and Genotypic Testing for Identification of *Streptococcus pneumonia* and Description of *Streptococcus pseudopneumoniaesp.J.Clin.Microbiol*.2004; Vol.42 (N°10): 4686-4696.DOI: 10.1128/JCM.42.10.4686–4696
10. Llull, D., Lopez, R., Garcia, E. Characteristic Signatures of the *lytA*Gene Provide a Basis for Rapid and Reliable Diagnosis of *Streptococcus pneumoniae*Infections. *J.Clin.Microbiol*.2006; Vol44 (N°4): 1250-1256.doi:10.1128/JCM.44.4.1250–1256
11. Corless, C.E., Guiver, M., Borrow, R., Edwards-Jones, V., Fox, A.J., and Kaczmarski, E.B. Simultaneous detection of *Neisseria meningitidis*, *Haemophilusinfluenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. *J.Clin.Microbiol*.2001; 39: 1553–1558.
12. Parent du Châtelet, I., Traoré, Y., Gessner, B.D., Antignac, A., Naccro, B., Njanpop-Lafourcade, B-M., Ouedraogo, M.S., Tiendrebeogo, S.R., Varon, E., Taha, M.K.Bacterial Meningitis in Burkina Faso: Surveillance Using Field-Based Polymerase Chain Reaction Testing. *Clin. Infect. Dis*. 2005; 40:17-25
13. Traoré, Y., Tameklo, T.A., Njanpop-Lafourcade, B-M., Lourd, M., Yaro, S., Niamba, D., Drabo, A., Mueller, J.E., Koeck, J-L., Gessner, B.D.Incidence, Seasonality, Age Distribution,and Mortality of Pneumococcal Meningitisin Burkina Faso and Togo. *Clin.Infect. Dis*. 2009; 48:S181-189
14. World Health Organization (WHO/V&B/03.01). Recommended standards for surveillance of selected vaccine-preventable diseases, the Department of Immunization, Vaccines and Biologicals thanks the donors whose unspecified financial support has made the production of this document possible, Geneva.2003; pp1-62.
15. Carvalho, M. da G.S., Tondella, M.L., Mc Caustland, K., Mc Gee, L.W.L., Mayer, L.W., Steigerwalt, A., Whaley, M., Facklam, R.R., Fields, B., Carlone, G., Ades, E.W., Dagan, R., Sampson, J.S., Evaluation and Improvement of Real-Time PCR Assays Targeting *lytA*, *ply*, and *psaA*Genes for Detection of Pneumococcal DNA. *J.Clin.Microbiol*. 2007; Vol. 45 (N°8)2460-2466. DOI: 10.1128/JCM.02498-06.
16. Sheppard, C.L., Harrison, T.G., Morris, R., Hogan, A., George, R.C.Autolysin-targeted Light Cycler assay including internal process control for detection of *Streptococcus pneumoniae* DNA in clinical samples. *J. Med.Microbiol*.2004; 53:189-195
17. Mc Avin, J.C., Reilly, P.A., Roudabush, R.M., Barnes, W.J., Salmen, A., Jackson, G.W., Beninga, K.K., Astorga, A., Mc Cleskey, F.K., Huff, W.B., Niemeyer, D., Lohman, K.L.Sensitive and Specific Method for Rapid Identification of *Streptococcus pneumoniae*Using Real-Time Fluorescence PCR. *J.Clin.Microbiol*.2001; Vol.39

(N°10): 3446-3451.DOI: 10.1128/JCM.39.10.3446–3451

18. Sacchi, C.T., Fukasawa, L.O., Goncalves, M.G., Salgado, M.M., Shutt, K.A., Carvalhanas, T.R., Ribeiro, A.F., Kemp, B., Gorla, M.C.O., Albernaz, R.K., Marques, E.G.L., Cruciano, A., Waldman, E.A., Brandileone, M.C.C., Harrison, L.H., Sao Paulo RT-Surveillance Project Team.Incorporation of Real-Time PCR into Routine Public Health Surveillance of Culture Negative Bacterial Meningitis in Sao Paulo, Brazil. *PLoSONE.* 2011; 6(6): e20675. doi:10.1371/journal.pone.0020675
19. Tzanakaki, G., Tsopanomichalou, M., Kesanopoulos, K., Matzourani, R., Sioumala, M., Tabaki, A., Kremastinou, J.Simultaneous single-tube PCR assay for the detection of *Neisseria meningitidis, Haemophilusinfluenzae* type b and *Streptococcus pneumoniae*. *Clin. Microbiol. Infect.*2005; 11:386-390
20. Failace, L., Wagner, M., Chesky, M., Scalco, R., Jobim, L.F.Simultaneous detection of *Neisseria meningitidis, Haemophilusinfluenzae*and *Streptococcus* sp. by polymerase chain reaction for the diagnosis of bacterial meningitis. *Arq.Neuropsiquiatr*.2005; 63(suppl 4):920-924
21. Abdeldaim, G.M.K., Stralin, K., Korsgaard, J., Blomberg, J., Welinder-Olsson, C., Herrmann, B. Multiplex quantitative PCR for detection of lower respiratory tract infection and meningitis caused by *Streptococcus pneumoniae*, *Haemophilusinfluenzae* and *Neisseria meningitidis.BMC Microbiol*. 2010; 10:310
22. Kennedy, W.A., Chang, S-J., Purdy, K., Kilgore, P.E., Kim, J.S., Anh, D.D., Huong, P.L.T., Dong, B.Q., Tan, D.M., Clemens, J.D., Ward, J.I.Incidence of bacterial meningitis in Asia usingenhanced CSF testing: polymerase chain reaction, latex agglutination and culture. *Epidemiol. Infect*.2007; 135:1217-1226.
23. Ceyhan, M., Yildirim, I., Balmer, P., Borrow, R., Dikici, B., Turgut, M., Kurt, N., Aydogan, A., Ecevit, C., Anlar, Y., Gulumser, O., Tanir, G., Salman, N., Gurler, N., Hatipoglu, N., Hacimustafaoglu, M., Celebi, S., Coskun, Y., Alhan, E., Celik, U., Camcioglu, Y., Secmeer, G., Gur, D., Gray, S.A prospective study of etiology of childhood acute bacterial meningitis, Turkey. *Emerg. Infect. Dis.* 2008; 1089-1096
24. Rintamaki, S., Saukkoriipi, A., Salo, P., Takala, A., Leinonen, M.Detection of *Streptococcus pneumoniae* DNA by using polymerase chain reaction and microwell hybridization with Europium-labelled probes. *J.Microbiol.Meth*.2002; 50:313–318
25. Messmer, T.O., Sampson, J.S., Stinson, A., Wong, B., Carlone, G.M., Facklam, R.R., Comparison of four polymerase chain reaction assays for specificity in the identification of *Streptococcus pneumoniae. Diag.Microbiol. Infect. Dis*.2004; 49:249-254
26. Kawamura, Y., Whiley, R.A., Shu, S-E., Ezaki, T., Hardie, J.M.Genetic approaches to the identification of the mitis group within the genus *Streptococcus. Microbiol*.1999; 145:2605-2613
27. Morrison, K.E., Lake, D., Crook, J., Carlone, G.M., Ades, E., Facklam, R., Sampson, J.S.Confirmation of *psaA*in all 90 serotypes of *Streptococcus pneumoniae*by PCR and potential of this assay for identification and diagnosis. *J.Clin.Microbiol*.2000;Vol. 38 (N°1): 434-437
28. Messmer, T.O., Whitney, C.G., Fields, B.S. Use of polymerase chain reaction to identify pneumococcal infection associated with hemorrhage and shock in two previously healthy young children. *Clin. Chem*.19997; 43 *(6 Pt1)*:930-935
29. Azzari, C., Moriondo, M., Indolfi, G., Massai, C., Becciolini, L., Martino, M., Resti, M.Molecular detection methods and serotyping performed directly on clinical samples improve diagnostic sensitivity and reveal increased incidence of invasive disease by *Streptococcus pneumoniae* in Italian children. *J.Med.Microbiol*.2008; 57:1205-1212

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INCIDENCE AND DISTRIBUTION OF MULTI-DRUG RESISTANT PATHOGENS FROM CLINICAL SAMPLES IN A TERTIARY HOSPITAL IN SOUTH-SOUTH NIGERIA.

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ABSTRACT

Background

Antibiotics have proven to be a dynamic category of drugs in the fight against infectious bacteria. However, antibiotic resistance is one of the greatest current challenges to the effective treatment of infections and there is every indication that antibiotic resistance will become an even greater challenge in the future.
Methodology
Ten clinical samples with varying frequencies were analyzed for bacterial growth, antibiotic susceptibility testing and multiple antibiotic resistances. The clinical samples includes; urine (42%), wound swab (21.33%), blood (10%), ear swab (9.33%), catheter tip (5.33%), endocervical swab (4.67%), high vaginal swab (HVS) (2.67%), urethral swab (2.67%), pus (1.33%) and antral aspirate (0.67%).
Results
A total of 150 bacterial isolates distributed among these ten (10) clinical samples were identified, of which *Staphylococcus aureus* (30%) was the most predominant, while *Klebsiella oxytoca, Citrobacter* spp. and *Streptococcus* spp. were the least (0.67%). These were tested for sensitivity against 9 antibiotics. The resistance rate observed was as follows; cefuroxime (93%), ceftazidime (87%), gentamycin (79%), augmentin (70%), cloxacillin (67%), ofloxacin (54%), ciprofloxacin (51%), ceftriaxone (38%) and ocefix (34%). One hundred and forty-three (95.3%) of the isolates showed multiple resistance against 3 – 8 antibiotics. None was resistant to as few as 1 – 2 antibiotics.
Conclusion
The high susceptibility to some antibiotics such as ceftriaxone and ocefix could be a welcoming relief, since they can be used to address the problem of resistance in this area. There is need for nationwide surveillance programme to monitor microbial trends and antimicrobial resistance patterns in Nigeria.

Key words: multi-drug resistant, clinical samples, *Staphylococcus aureus*, ocefix.

INCIDENCE ET REPARTITION DES AGENTS PATHOGENES MULT-RESISTANTS AUX ANTIBIOTIQUES ISOLES DES ECHANTILLONS CLINIQUES A L'HOPITAL DU SOIN TERTIAIRE DANS LE SUD DU NIGERIA

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Contexte
Les antibiotiques se sont démontrés être une catégorie dynamique des médicaments pour lutter contre les infections bactériennes. Cependant, la résistance aux antibiotiques est l'un des grands défis actuels pour le traitement efficace des infections et tout porte à croire que la résistance aux antibiotiques devient un défi encore plus grand à l'avenir.
Méthodologie
Dix échantillons cliniques avec leurs grandes fréquences ont été analysés par la culture bactériennes, le teste de sensibilité aux antibiotiques et le teste de la multirésistance aux antibiotiques. Les échantillons cliniques sont constitués de l'urine (42%), de pus de la plaie (21,33%), de sang (10%), de prélèvement d'oreille (9,33%), d'extrémité du cathéter (5,33%), de prélèvement d'endocervical (4,67%), de prélèvement vaginal (2,67%), de prélèvement urétral (2,67%), de pus (1,33%), de ponctionantrale(0,67%).
Résultats
Au total, 150 souches bactériennes réparties parmi les dix (10) échantillons cliniques ont été identifiées, parmi lesquelles *Staphylococcus aureus* (30%) était le plus prédominant, alors que *Klebsiellaoxytoca,Citrobacter*spp et*Streptococcus*spp étaient les moindres (0,67%). Ils ont été testés pour la sensibilité de 9 antibiotiques. Le taux de résistance observé était le

suivant: céfuroxime (93%), ceftazidime (87%), gentamicine (79%), augmentin (70%), cloxacilline (67%), ofloxacine (54%), ciprofloxacine (51%), ceftriaxone (38%) etocefix (34%). Cent-quarante-trois (95,3%) d'isolats ont montré la multi-résistance contre 3 à 8 antibiotiques. Aucune souche n'a été résistante à moins de 1 à 2 antibiotiques.

Conclusion

La forte sensibilité de certains antibiotiques tels que la ceftriaxone et l'ocefix pourraient être un ouf de soulagement, car ils peuvent être utilisés pour résoudre le problème de la résistance dans cette région. Il est nécessaire pour le programme national de surveillance pour suivre les tendances microbiennes et les situations de résistance aux antimicrobiens au Nigeria.

Mots clés: multi-résistance aux antibiotiques, échantillons cliniques, *Staphylococcus aureus*, ocefix.

INTRODUCTION

Antimicrobial resistance is a well known clinical and public health problem [1]. Over the last 60yrs, bacteria in particular, those pathogenic for humans have evolved toward antimicrobial drug resistance. This evolution has 2 key steps; emergence and dissemination of resistance [2]. Antimicrobial resistance in nosocomial infections is increasing with both morbidity and mortality greater than when infection is caused by drug sensitive organisms. These infections are opportunistic and microorganisms of low virulence can cause diseases in hospitalized patients whose immune mechanisms are impaired. The outcome is that many antibiotics can no longer be used for the treatment of infections caused by such organisms and the threat to the usage of other drugs increases [3, 4].

The widespread use of broad- spectrum antibiotics, immunocompromised patients, and exogenous transmission of bacteria, usually by hospital personnel has led to the emergence of nosocomial infections caused by drug resistant microbes [5, 6]. Available therapeutic options for antibiotic resistant organisms are severely limited, as these organisms frequently display a multi-drug resistant phenotype [7, 8, and 6]. Multi-drug resistance (MDR) and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease scourge [9]. However, strategies for addressing antimicrobial drug resistance stress the need for new drug [10] and yet the rate of drug development is in decline [11, 12].

Several investigators revealed that the most frequently reported bacterial pathogens from clinical sources with multi-drug resistance have been *E. coli, S. aureus, P. aeruginosa, K. pneumoniae, Proteus* spp., *Acinetobacter* spp., *Enterococcus* spp., coagulase negative staphylococci (CONS), etc.[13, 14, 15 and 16].

As the proliferation of multi-drug resistance pathogens continue unavoidably in the hospital settings, within and around us, it is imperative that their resistance trend be put under check through intensive research and antibiotic surveillance. Therefore, this current study reports on the incidence and distribution of multi-drug resistant bacteria pathogens from clinical samples in a tertiary hospital in south-south Nigeria.

MATERIAL AND METHODS

Sample collections

One hundred and fifty clinical samples classified into ten groups were obtained from routine section in Medical Microbiology Laboratory of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The sources of the samples were as follows: urine (42%), wound swab (21.33%), blood (10%), ear swab (9.33%), catheter tip (5.33%), endocervical swab (4.67%), high vaginal swab (2.67%), urethral swab (2.67%), pus (1.33%), and antral aspirate (0.67%). The samples were analysed for bacterial growth within 1-2 hrs after collection.

Identification of bacterial isolates

All the samples were plated on Blood agar, MacConkey agar and replicated on Mannitol salt agar (oxoid, England). The inoculated plates were incubated at 37°C for 24h. Identification of bacterial isolates was done on the basis of their cultural and standard biochemical characteristics. [17]. The isolates were sub-cultured on nutrient agar slants periodically to maintain pure culture.

Antibiotic Susceptibility Testing

Isolates were tested for antimicrobial susceptibility testing by the standard disc diffusion method. Standard inoculums adjusted to 0.5 McFarland was swabbed on Mueller Hinton agar and was allowed to soak for 2 to 5 minutes. After that the appropriate antibiotic disc were aseptically placed on the agar surface using sterile forceps and the plates were incubated at 37^0C for 24hrs. Commercially available antimicrobial discs used included: ciprofloxacin (30μg), ceftriaxone (10μg), ofloxacin (25μg), augmentin (25μg), cefuroxime (30μg), gentamycin (10μg), ceftazidime (30μg), cloxacillin (10μg), and ocefix (10μg). The degree of susceptibility of the test isolates to each antibiotic was interpreted as either sensitive (S) or resistant (R) according to National Committee for Clinical Laboratory Standards [18].

Statistical analyses:

The Chi-square goodness of fit test was used to test for significant differences in the data obtained. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and non- significance difference was defined when $(p \leq 0.05)$ and $(p \geq 0.05)$ respectively.

RESULTS

Figure 1 showed the sources of 10 clinical samples used in the study. Urine (42%) was the most predominant clinical sample, followed by wound swab (21.3%) and blood (10%); while the least clinical sample was antral aspirate (0.7%), closely followed by pus (1.3%) and HVS (2.7%).

The isolates were confirmed as *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis*, coagulase negative staphylococci (CoNS), *Citrobacter* spp., *Alcaligenes* spp., *Enterobacter* spp., *Klebsiella oxytoca* and *Streptococcus* spp. Of these isolates, 57(38%) were Gram positive and 93(62%) were Gram negative. *Staphylococcus aureus* was the most common Gram positive isolates (79%), while *Klebsiella pneumoniae* was the most common Gram negative isolates (31%). *Staphylococcus aureus* 45(30%) was the most predominant isolate, followed by *Klebsiella pneumoniae* 28(18.7%), while *Citrobacter* spp., *Klebsiella oxytoca* and *Streptococcus* spp. were the least with 1(0.7%) each (Table I). *Staphylococcus aureus* had their highest occurrence from urine samples. *Pseudomonas aeruginosa* had its highest incidence in wound swab, while *Klebsiella pneumoniae* was the most common organism isolated from catheter tip and blood. The result also showed that *Staphylococcus aureus* and CoNS were the most predominant organisms in endocervical swab, while *S. aureus and P. aeruginosa* were the most occurring isolates from ear swab.

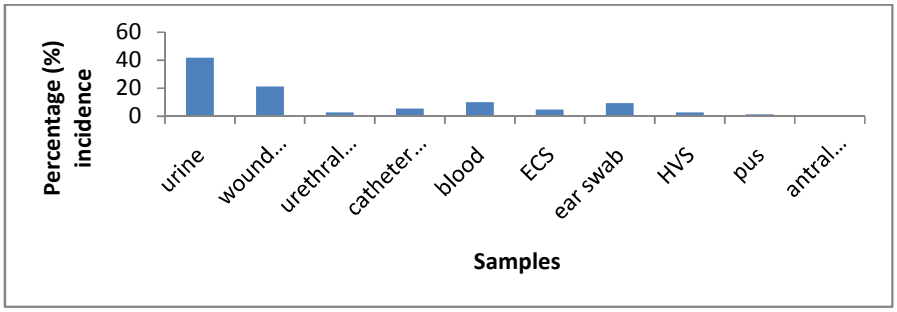

Figure 1: Percentage incidence of clinical samples.

Key: ECS = Endocervical swab, HVS = high vagina swab

TABLE I: PREVALENCE OF BACTERIA ISOLATES FROM CLINICAL SAMPLES (%)

CLINICAL SAMPLES	SA	EC	KP	KO	CONS	PA	PM	CS	AS	ES	SS
Urine	21(46.67)	15(57.69)	12(42.85)	-	4(36.36)	-	4(33.33)	-	6(75)	1(50)	-
WS	6(13.33)	7(26.92)	2(7.14)	-	-	11(73.33)	3(25)	-	2(25)	1(50)	-
US	4(8.89)	-	-	-	-	-	-	-	-	-	-
CT	-	-	6(21.43)	-	-	-	2(16.67)	-	-	-	-
Blood	4(8.89)	2(7.69)	5(17.86)	1(100)	1(9.09)	-	1(8.33)	1(100)	-	-	-
ECS	3(6.67)	-	1(3.57)	-	3(27.27)	-	-	-	-	-	-
Ear swab	4(8.89)	-	2(7.14)	-	2(18.18)	4(26.67)	2(16.67)	-	-	-	-
HVS	1(2.22)	2(7.69)	-	-	-	-	-	-	-	-	1(100)
Pus	2(4.44)	-	-	-	-	-	-	-	-	-	-
AS	-	-	-	-	1(9.09)	-	-	-	-	-	-
Total	45(30)	26(17.33)	28(18.67)	1(0.67)	11(7.33)	15(10)	12(8)	1(0.67)	8(5.33)	2(1.33)	1(0.67)

KEY: SA = *Staphylococcus aureus*, EC: *Escherichia coli*, KP = *Klebsiella pneumoniae*, KO = *Klebsiella oxytoca*, CONS = Coagulase negative Staphylococcus PA = *Pseudomonas aeruginosa*, PM= *Proteus mirabilis*, CS = *Citrobacter* spp., AS = *Alcaligens* spp., ES = *Enterobacter* spp., SS = *Streptococcus* spp., WS = Wound swab, US = Urethral swab, CT = catheter tip, ECS = Endocervical swab, HVS = High vagina swab, AS = Antral aspirate

Table II showed the antibiogram of the isolates. Resistance by all the isolates to cefuroxime was the highest (92.7%), followed by ceftazidime (86.7%), gentamycin (79.3%), augmentin (70%), cloxacillin (66.7%), ofloxacin (54%) and ciprofloxacin (51.3%). The least resistance was to ocefix (34%). Resistance

TABLE II: ANTIBIOGRAM OF ISOLATES (%)

Antibiotics	No.sensitive	No.resistance
Ciprofloxacin	73(48.7)	77(51.3)
Ceftriaxone	93(62)	57(38)
Ofloxacin	69(46)	81(54)
Augmentin	44(29)	106(70)
Cefuroxime	11(7.3)	139(92.7)
Gentamycin	31(20.7)	119(79.3)
Ceftazidime	20(13.3)	130(86.7)
Cloxacillin	50(33.3)	100(66.7)
Ocefix	99(66)	51(34)

against cefuroxime by all the isolates were not significantly different from that of ceftazidime and gentamycin ($p > 0.05$), but were significantly higher than other antibiotics ($p < 0.05$). Similarly, resistance against ocefix by all the isolates were not significantly different from that of ceftriaxone and ciprofloxacin ($p > 0.05$), but were significantly lower than that of other antibiotics ($p < 0.05$). The result further showed significant correlation between cefuroxime and ceftazidime ($p < 0.01$).

TABLE III: NO. OF ORGANISMS WITH MULTIPLE ANTIBIOTIC RESISTANCE (%)

No. of organisms	Percentage of organisms	No. of antibiotics
0	0	1
0	0	2
7	4.7	3
21	14.0	4
29	19.3	5
40	26.7	6
39	26.0	7
14	9.3	8
150	100	

Of the 150 isolates obtained, 143(95.3%) of them were multi-drug resistant. Seven(4.7%) of the isolates were resistant to 3 antibiotics, 21(14%) were resistant to 4 antibiotics, and 29(19.3%) were resistant to 5 antibiotics. While 40(26.7%), 39(26%), and 14(9.3%) of the isolates were resistant to 6, 7, and 8 antibiotics respectively. Ninety three (62%) of the isolates were resistant to at least 6 antibiotics (Table III).

DISCUSSION

The result of this study shows that the most predominant clinical sample was urine. This result was in conformity with the works of some researchers who in their studies also reported that urine was the most common clinical sample encountered [13, 19 and 20].

The observation in this study that *Staphylococcus aureus* was the predominant organism from nosocomial samples examined is in support with reports from other researchers [21, 22, 23, 24 and 25]. It however contradicts reports from some researchers who reported other organisms to be most predominant. Such organisms include *E. coli* [26, 27 and 20], *Klebsiella pneumoniae* [19], *and Staphylococcus epidermidis* [6]. The high incidence of *S. aureus in* this study could be as a result of its minimal growth requirements, ability to survive long in most unfavorable environments and in a susceptible host. It could also be due to the virulent nature of the organism, which gives it the ability to overcome body defense mechanisms and resistance to antibiotics [24]. Urinary tract instrumentation and presence of indwelling catheter increase the risk of *S. aureus* carriage in the urinary tract [28].

The finding in this study that *Pseudomonas aeruginosa* had its highest occurrence in wound swab is contrary to the findings of Mordi and momoh [25] who reported that *S. aureus* was the most predominant organism in wound swabs.

The observation in this work that *K. pneumoniae* was the most predominant organism from catheter tips and blood is contrary to Iffat *et al* [*20*], who reported *S. aureus* to be the most predominant isolate instead. Their report that the most predominant isolate from pus was *E. coli* is also contrary to the observation in this study which revealed *S. aureus* to be the predominant organism from pus

The most predominant Gram negative organism isolated in this study was *K. pneumoniae*. This result disagrees with that of some researchers who reported *E. coli* to be the predominant Gram negative organisms in their samples [20, 26 and 27]. Also, Mordi and Momoh, [25] reported that *Proteus* spp. was the most predominant Gram negative isolate in their study. Variations in the types of organisms isolated could be attributed to factors such as environmental difference, media, culturing method, time and method of sampling. Period of transportation and storage before culturing can also be causative factors.

The result of this study revealed very high resistance of all isolates towards cefuroxime

(92.7%). This result is contrary to the findings of Yah and Eghafona [29], who reported very low resistance of their diarrhoeal isolates towards cefuroxime. It however, agrees with the work of Adeleke *et al*. [30], who reported high resistance towards cefuroxime (81.8%) by Gram negative organisms. Their reported high resistance against ceftriaxone however contradicts the observation in this study in which very low resistance to same antibiotic was observed. Iffat *et al*. [20], in their work reported high resistance rate among Gram negative organisms against all generations of cephalosporin antibiotics as well as β-lactam/β-lactamase inhibitors. This study in contrast observed low resistance rate towards ceftriaxone (38%) and ocefix (34%), both of which are cephalosporins.

The contrasting antibacterial activity of ceftriaxone (38% resistance) and cefuroxime (92.7% resistance) in this study agrees with the previous reports of a better activity for ceftriaxone than cefuroxime [31, 32 and 33]. Nevertheless, the equally contrasting multiple drug resistance against cefuroxime, ceftazidime, ceftriaxone and ocefix (all cephalosporins) confirm the alarm raised by Paul *et al*.[34], on the alarming rate of resistance to cephalosporins by the hospital strains especially Gram negative bacteria and more so with the advent of the extended spectrum β-lactamases producers [35, 36]. This confirms a report that in Nigeria, β-lactams are the most frequently prescribed antibiotics especially in Gram negative infections and selective pressure exerted by the use of these β-lactam drugs have resulted in the strains producing the extended spectrum β-lactamases enzymes [37].

The result of this study further showed that all the isolates were highly resistant against gentamycin (79.3%), augmentin (70%), cloxacillin (66.7%), ofloxacin (54%), and ciprofloxacin (51.3%). Similar resistance patterns were reported by other workers [19, 26]. High resistance toward ciprofloxacin by *Klebsiella pneumoniae* and low resistance against ciprofloxacin by CoNS and *Pseudomonas aeruginosa* as observed in this study had also been reported by Iffat *et al*. [20].

Almost all the pathogens isolated in this study have also been shown to cause different forms of nosocomial infections [7, 8, 38, 39, and 40]. *S. aureus* exhibits remarkable versatility in their behaviour towards antibiotics [41]. Outbreaks of *S. aureus* resistant to β-lactam antibiotics have been frequently associated with devastating nosocomial infections [6, 42 and 43].

Klebsiella pneumoniae (and some related species) is an important opportunistic Gram-negative rod pathogen involved in the outbreaks of nosocomial infections, meningitis, lower respiratory, urinary tract and burn wound infections. The members of this genus have also been linked to epidemics of diarrhea, because some strains appear to have acquired plasmids from *E. coli* (that code for the heat labile and heat stable enterotoxins [44, 45].

Pseudomonas aeruginosa is a ubiquitous organism, an opportunistic pathogen, and can cause a wide range of infections including bacterial meningitis, endocarditis, Otitis media [46] urinary tract infection [47] and osteomyelitis [48]. *Pseudomonas aeruginosa* is notorious for its resistance to antibiotics and is therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistance to many antibiotics due to the permeability barrier afforded by its outer membrane lipopolysaccharide (LPS). Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics [49].

Escherichia coli is an important opportunistic pathogen that has shown an increasing antimicrobial resistance to most antibiotics [50, 51]. Intestinal strains of *E. coli* are primary cause of urinary tract infections, septicaemia, diarrhoea, neonatal meningitis and nosocomial infections. Individuals who are debilitated or have other predisposing factors are at much risk for infection than healthy person [52]. Antibiotic resistance was high among *E. coli* strains, which emphasize the need for judicious use of antibiotics. Certain virulence factors like hemolysin production and presence of fimbriae in the strain may be associated with its virulence especially urovirulence [53].

Coagulase negative staphylococci (CoNS) especially *Staphylococcus epidermidis* is a major cause of nosocomial infections because of its ability to form biofilms on the surface of medical devices. Bacterial biofilms are inherently resistant to antibiotics and host defences, and this could explain the reason for the high resistance seen in the strains isolated in this study [6, 54, and 55].

Eight (8) strains of *Alcaligenes* spp., were isolated in this study, and were 100% resistant to cefuroxime, augmentin and ceftazidime. The strains were also highly resistance to ofloxacin, gentamycin and ciprofloxacin. *Alcaligenes* spp. is generally considered non-pathogenic but can occur as an opportunistic pathogen in urinary tract infection [56]. Some species of *Alcaligenes* are potential causes of chronic pulmonary diseases in patients with cystic fibrosis [57].

High rates of drug resistance were found in most of the isolates studied. In developing countries like Nigeria, self medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospitals when they are unable

to treat themselves. On admission, the community acquired resistant strains exchange genetic material with nosocomial isolates resulting in the emergence of 'superbugs' that could cause difficult to treat infections [6, 58]. Inappropriate practice like misuse and abuse of antibiotics and unskilled practitioners can also lead to emergence of resistance in bacteria. Expired antibiotics, counterfeit drugs, inadequate hospital infection control measures can as well promote the development of resistance in clinical isolates [6, 59].

Conclusion

It is apparent from the results of the antibiogram that hospitals could be a potential reservoir of nosocomial antibiotic resistant pathogens. The high incidence of antibiotic resistant strains isolated from clinical samples of patients in University of Benin Teaching Hospital (UBTH) is worrisome, and need to be addressed properly. We highly recommend the development of infection control programmes for the surveillance of nosocomial infections and epidemiologic typing of clinical isolates in hospitals in order to check the emergence and spread of antibiotic resistant infections in patient.

REFERENCES

1. Oteo J, Campos J, Baquero F. Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European antimicrobial resistance surveillance system. *Journal of Antimicrobial Chemotherapy, 2002;* 50: 945-952.
2. Courvalin P. Antimicrobial drug resistance: "prediction is very difficult, especially about the future". *Emerging Infectious Disease,* 2005; 11: 1503-1506.
3. Courvalin P. Evasion of antibiotic action by bacteria. *Journal of Antimicrobial Chemotherapy,* 1996; 37: 855-869
4. Chopra I. Research and development of antibacterial agents. *Current Opinion* on *Microbiology,* 1998; 1: 495-501.
5. Courvalin P. and Weber JT. Antimicrobial drugs and resistance. *Emerging Infectious Disease*, 2005; 11: 791-797.
6. Chikere CB, Chikere BO, Omoni VT. Antibiogram of clinical isolates from a hospital in Nigeria. *African Journal of Biotechnology*. 2008; 7(24): 4359-4363.
7. Moland ES, Hanson ND, Black JA, Hossain A, Song W, Thomson KS. Prevalence of newer β-lactamases in Gram-negative clinical isolates collected in the United States from 2001 to 2002. *Journal of Clinical Microbiology*, 2006; 44: 3318-3324.
8. Lewis JS, Herraera M, Wickes B, Patterson JE, Jorgensen JH. First report of the emergence of CTX-M-Type extended-spectrum β-lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. *Antimicrobial Agents and Chemotherapy*. 2007; 51: 4015-4021.
9. Okonko IO, Fajobi EA, Ogunnusi TA, Ogunjobi AA, Obiogbolu CH. Antimicrobial chemotherapy and sustainable development: the past, the current trend, and the future. *African Journal of Biomedical Research*, 2008; 11(3): 235-250.
10. World Health Organization. Best infection control practices for skin-piercing intradermal, subcutaneous, and intramuscular needle injections. WHO/BCT/DCT/01.02, 2001.
11. Power JH. Antimicrobial drug development – the past, present and future. *Clinical Microbial Infection, 2004;* 10: 23-31.
12. Metlay JP, Powers JH, Dudley MN, Christiansen K, Finch RG. Antimicrobial drug resistance, regulation and research. *Emerging Infectious Disease,* 2006; 12(20: 183-190.
13. Patwardhan RB, Dhakephalkar PK, Niphadkar KB, Chopade BA. A study on nosocomial pathogens in ICU with special reference to multiresistant *Acinetobacter baumannii* harbouring multiple plasmids. *Indian Journal of Medical Research*, 2008; 128: 178-187.
14. McCraig LF, McDonald LC, Mandal S, Jermigan DB. *Staphylococcus aureus* associated skin and soft tissue infections in ambulatory care. *Emerging Infectious Disease*, 2006; 12: 1715-1723.
15. Pitout JDD, Nordman P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended – spectrum β-lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy*, 2005; 56: 52-59.
16. Usha BN, Jalil JB, Vaishali BN, Roshan D. R-plasmid of nosocomial *P. aeruginosa* act as broad spectrum resistance. *Developmental Microbiology and Molecular Biology*, 2010; 1(1): 37-44.
17. Washington CW, Stephen DA, Williams MJ, Elomer WK, Gary WP, Paul CS, Gail LW.
Koneman's Colour Atlas and Textbook of Diagnostic Microbiology, 6th edn. Lippincott Williams and Wilkins, Philadelphia, USA; 2006.
18. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility testing: Twelfth informational supplement. NCCLS document M100-S12. PA, USA. 2002.

19. Iheanyi OO, Femi AS, Timothy AA, Ayoteju AO, Tolulope AO, Joan E. Incidence of multi-drug resistance (MDR) organisms in Abeokuta, South Western Nigeria. *Global Journal of Pharmacology*, 2009; 3(2); 69-80.
20. Iffat J, Hafeez R, Anwar SM. Antibiotic susceptibility pattern of bacterial isolates from patients admitted to a tertiary care hospital in Lahore. *Biomedical*, 2011; 27: 19-23.
21. Sule Odu AO. Bacterial flora of surgical wounds. *Tropical Journal of Obstetrics and Gynecology*. 1991; 9(2): 41.
22. Akerele J and Ahonkhai IA. Urinary pathogen and antibacterial susceptibility. A retrospective study of private diagnostic laboratory in Benin City Nigeria. *Journal of Medical Laboratory Science*, 2000; 9: 47-48.
23. Ahmed BM and Kudi AA. Chronic supperative otitis media in Gombe, Nigeria. *Journal Surgical Research*, 2003; 5: 3-4.
24. Akortha EE and Ibadin OK. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amongst patients with urinary tract infection (UTI) in UBTH Benin City, Nigeria. *African Journal Biotechnology*, 2008; 7(11): 1637-1640.
25. Mordi RM and Momoh MI. Incidence of *Proteus* species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. *African Journal of Biotechnology*, 2009; 8(5): 725-730.
26. Aminizadeh Z. and Kashi MS. Prevalence of multi-drug resistance and pandrug resistance among multiple Gram negative species; experience in one teaching hospital, Tehran, Iran. *International Research Journal of Microbiology*, 2011; 2(3): 090-095.
27. Manikandan S, Ganesapandian S, Manoj S, Kumaraguru AK. Antimicrobial susceptibility pattern of urinary tract infection causing human pathogenic bacteria. *Asian Journal of Medical Sciences*, 2011; 3(2): 56-60.
28. Coll PP, Crabtree BF, O'Connor PJ, Klenzak S. Clinical risk factors for methicillin-resistant *Staphylococcus aureus* bacteriuria in a skilled-care nursing home. *Archives of Family Medicine*, 1994; 3:357–60.
29. Yah CS and Eghafona NO. Plasmids: a vehicle for rapid transfer of antibiotic resistance markers of *Salmonella* species in animals. *International Journal of Integrative Biology*, 2008; 2(1): 55-61
30. Adeleke OE, Inwezerua C, Smith SI. Plasmid- mediated resistance of some Gram-negative bacteria species to brands of cefuroxime and ceftriaxone. *Scientific Research and Essay*. 2010; 5(14): 1813-1819.
31. Foye WO, Lemke TL, Williams DA. Cephalosporins. *Principles of Medical Chemistry*. 4th edn Media, PA, Williams and Wilkins, Baltimore, MD. 1995; 782.
32. Williams KN. (1995). Anti-infectives in Remingtons: the science and practice of pharmacy (Eds: Genaro, AR., Medwick, T., Edward GR., Zink, GL., Schwartz, JD., Chase, DG., Hussar, AD., Manderosian, DA., Hanson, GR., White, HS) 19th edn. Mark publishing company, Easton, Pennsylvania, 18042. 1995; pp. 1291-1297.
33. Chambers HF, Hadley WK, Jawetz E. (1998). Beta-lactam antibiotics and other inhibitors of cell wall synthesis. Basic and Clinical Pharmacology (katzung, BG ed.)7th edition Appleton and Lange Stamford. Connecticut. 1998; 725-741.
34. Paul S, Bezbaruah MK, Gosh AC. Multiple antibiotic resistance (MAR) and its reversion in *Pseudomonas aeruginosa*. *Letters in Applied Microbiology*, 1997; 24: 169-171.
35. Butaye P, Cloeckaert A, Schwar ZS. Mobile genes coding for efflux-mediated antimicrobial resistance in Gram-positive and Gram-negative bacteria. *International Journal of Antimicrobial Agents*, 2003 22: 205-210.
36. Soge OO. Molecular basis of multidrug resistance in uropathogenic *Klebsiella pneumoniae* from south western Nigeria. A Ph.D. thesis in the department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria, 2007.
37. Aibinu I, Odugbemi T, Mee BJ. Extended spectrum β-lactamases in isolates of *Klebsiella* spp. and *Escherichia coli* from Lagos, Nigeria. *Nigeria Journal of Health and Biomedical Science*, 2003; 2(2): 53-60.
38. Leavitt A, Navon-venezia S, Chmeinitsky I, Schwaber MJ, Carmeli Y. Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli hospital. *Antimicrobial Agents and Chemotherapy*, 2007; 51(8): 3026-3029.
39. Lockhart SR, Abramson MA, Beekman SE, Gallagher G, Riedel SR, Diekma DJ, Quinn JP, Doern GV. Antimicrobial resistance among Gram-negative bacilli as causes of infections in intensive care unit patients in the United States between 1993 and 2004. *Journal of Clinical Microbiology*, 2007; 45: 3352-3359.
40. Mendonca N, Leitao J, Manageiro V, Ferreira E, Canica M. The antimicrobial resistance surveillance program in Portugal. Spread of extended spectrum β-latamase CTX-M- producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. *Antimicrobial Agents and Chemotherapy*, 2007; 51(6): 1946-1955.

41. Obiazi HAK, Nmorsi OPG, Ekundayo AO, Ukwandu NCD. Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* from clinical isolates grown at 37^0C and 44^0C from Irrua, Nigeria. *African Journal of Microbiology Research*, 2007; 1 (5): 057-060
42. Depardieu F, Podglajen I, Leclercq R, Collatz E, Courvalin P. Modes and modulations of antibiotic resistance gene expression. *Clinical Microbiology Review*, 2007; 20(1): 79-114.
43. Buhlmann M, Bogli-Stuber K, Droz S, Muhlemann K. Rapid sceering for carriage of methicillin-resistant *Staphylococcus aureus* by PCR and associated costs. *Journal Clinical Microbiology*, 2008; 46(7): 2151-2154.
44. Ewing WH. Identification of Enterobacteriaceae (4^{th} edtion). Elsevier, Netherlands. 1986
45. Sheikh AR, Afsheen A, Sadia K, Abdul W. (2003). Plasmid borne antibiotic resistance factors among indigenous *Klebsiella*. *Pakistan Journal*. 2003; 35(2): 243-248.
46. Lee JC, Remtulla RA, Stevens GR, Zhang M, Antonelli PJ. Preoperative antibiotic steroid therapy and hearing loss caused by semicircular canal transaction in *Pseudomonas* Otitis media. *Otolarynal Head and Neck Surgery,* 2005; 132: 896-901.
47. Harjai K, Mittal R, Chhibber S, Sharma S. Contribution of Tamm-Horsfall protein to virulence of *Pseudomonas aeruginosa* in urinary tract infection. *Microbes and Infection*, 2005; 7: 132-137.
48. Andonian S, Rabah DM, Aprikan AG. *Pseudomonas aeruginosa* sacrolitis and osteomylitis of pelvic bones after radial prostatectomy. *Urology,* 2002; 60: 698-700.
49. Ryan KJ and Ray CG. (editors). *Sherries Medical Microbiology*, 4^{th} ed., McGraw Hill. ISBN 0838585299, 2004.
50. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern, GV. Evidence for transfer of CYM-2 AMPc-lactamase plasmids between *E. coli* and *Salmonella* isolates food, animals and humans. *Antimicrobial Agents and Chemotherapy*, 2001; 45(10): 2716-2722.
51. Miranda S, David MG, Peter JC. Evolution of multi-resistance plasmids in Australia clinical isolates of *Escherichia coli*. *Microbiology*, 2004; 150: 1539-1546.
52. Lisa AS and Rodgers AI. Essentials of diagnostic microbiology. Delmar Publishers. 1999; 186-205.
53. Mandal P, Kapil A, Goswani K, Das B, Dwivedi SN. Uropathogenic *Escherichia coli* causing UTI's. *Indian Journal of Medical Research*, 2001; 114: 207-211.
54. Cloete TE. Resistance mechanisms of bacteria to antimicrobial compounds. *International Biodeteriology and Biodegradation*, 2003; 51: 277-282.
55. Villain-Guillot P, Gualtieri M, Bastide L, Leonetti JP. In vitro activities of different inhibitors of bacterial transcription against *Staphylococcus epidermidis* biofilm. *Antimicrobial Agents and Chemotherapy*, 2007; 51(9): 3117-3121.
56. Quinn RJ. Clinical problems posed by multi-drug resistance non-fermenting Gram-negative pathogens. *Clinical Infectious Disease,* 1998; 27: 17-124.
57. Salman L. Identification and antimicrobial susceptibility of *Alcaligenes xylosoxidans* isolated from patients with cystic fibrosis. *Journal of Clinical Microbiology*, 2001; 39(11): 3942-3945.
58. Mulvey MR, Bryce E, Boyd D, Ofner-Agostini M, Christianson S, Simor AE, Paton, S, the Canadian hospital epidemiology committee of the Canadian nosocomial infection surveillance program, Health Canada. Amber class A extended spectrum β-lactamase – producing *Escherichia coli* and *Klebsiella* spp. in Canada hospitals. *Antimicrobial Agents and Chemotherapy*, 2004; 48: 1204-1214.
59. Prescott LM, Harley JP, Klein DA. *Microbiology*. 6^{th} ed., McGraw-Hill, New York. 2005; 833-842.

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MICROBIOLOGICAL PROFILE OF ORAL INFECTIONS IN DIABETIC PATIENTS AND NON-DIABETIC CONTROLS IN SOUTHWEST, CAMEROON

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ABSTRACT

Background: Oral microbial flora is increasingly being incriminated in oral infections. There is paucity of information on the importance of aerobic oral flora in diabetes. The purpose of this study was to compare aerobic oral microbial flora in diabetics and non-diabetics and to relate these microbes with oral infections.

Materials and Methods: This study involved 154 diabetics and 111 non-diabetics aged 18 years and above. Oral washes were inoculated unto blood agar, chocolate agar, Mac Conkey agar and Sabouraud's agar and isolates were identified by standard biochemical tests. Oral exam was conducted by a Dentist to assess oral infections and oral health status of participants.

Results: Thirteen different genera of aerobic microbes were identified. The most prevalent microbes were *Streptococcus* sp (99.6 %), *Candida albicans* (17.0 %), *Serratia* Spp (7.2 %), other *Candida* Spp (6.8 %), Coagulase negative Staphylococci (CNS) (6.4 %) and *Klebsiella* Spp (5.7 %). *Candida* sp was more prevalent in diabetic patients than non-diabetics. Gram negative aerobic bacteria were significantly isolated from cases of dental caries.

Conclusion: The oral microbiological profile of diabetic patients was different from those of non-diabetics and aerobic Gram negative bacteria may play an important role in dental diseases in diabetic patients.

Keywords: Oral microbiological profile; oral infections; diabetes; Cameroon

PROFIL MICROBIOLOGIQUE DES INFECTIONS BUCCALES CHEZ LES PATIENTS DIABETIQUES ET TEMOINS NON DIABETIQUES DU SUD-OUEST, CAMEROUN

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RÉSUMÉ

Contexte: La flore microbienne orale est de plus en plus incriminée dans les infections buccales. Il existe peu d'informations sur l'importance de la flore buccale aérobie chez les diabétiques. Le but de cette étude était de comparer la flore microbienne aérobie orale chez les diabétiques et les non diabétiques et de déterminer le lien que ces germes ont avec les infections buccales.

Matériels et méthodes: Cette étude a porté sur 154 diabétiques et 111 non-diabétiques âgés de 18 ans et plus. Le liquide de lavage buccal a été inoculé sur des géloses au sang, au chocolat, de Mac Conkey et de Sabouraud respectivement, et les souches bactériennes ont été identifiées par des tests biochimiques standards. L'examen oral a été mené par un dentiste afin d'évaluer les infections buccales et l'état de santé bucco-dentaire des participants.

Résultats: Treize genre différents de microorganismes aérobies ont été identifiés. Les microbes les plus répandus étaient *Streptococcus sp* (99,6%), *Candida albicans* (17,0%), *Serratia spp* (7,2%), les autres espèces de *Candida* (6,8%), les staphylocoques à coagulase négative (SCN) (6,4%) et *Klebsiella spp* (5,7%). *Candida* spp était plus fréquent chez les patients diabétiques que chez les non-diabétiques. Les bactéries Gram négatives aérobies ont été considérablement isolées des cas de caries dentaires.

Conclusion: Le profil microbiologique oral des patients diabétiques était différent de ceux des non- diabétiques. Les bactéries Gram négatif aérobies peuvent jouer un rôle important dans les maladies dentaires chez les patients diabétiques.

Mots-clés: Profil microbiologique orale; infections buccales; diabète; Cameroun

INTRODUCTION

The microbial flora of the human oral cavity is highly diverse, consisting of mainly bacteria and fungi (1). The normal flora of the human mouth is mainly made up of streptococci and anaerobic Gram negative bacteria (1, 2). These microorganisms play an important role in preventing colonisation by pathogenic microbes; thereby maintaining the health of the oral cavity. It is known that disruption of the normal flora can trigger or influence the course of oral diseases (2). Apart from influencing the onset or course of oral infections, oral microbial flora is also associated with some systemic diseases (3, 4). Staphylococci and aerobic Gram negative bacteria are not endogenous flora but are considered transient colonisers of the oral cavity; from which they cause infections like pneumonia (5). Systemic changes such as disease, pregnancy and puberty are known to alter the microenvironment of the oral cavity and consequently influence the proportion and type of oral flora (2, 6). Diabetes has been associated with oral infections such as periodontitis (7, 8), dental caries (9), gingivitis (10) and candidiasis (11). The high prevalence of oral infections in diabetes patients has also been liked to poor oral hygiene (12). However, whether oral hygiene influences oral microbial colonisation is not fully elucidated. The southwest region of Cameroon is a rainforest zone and majority of the population depends on agriculture and pastoral activities for their livelihoods. Alcohol consumption is a normal practice of most inhabitants. In Cameroon like in most African countries, oral health is seen as a very low priority where the limited resources available to the health sector are directed towards life-threatening conditions such as HIV/AIDS, tuberculosis, and malaria. The few dental clinics in the Region are located in the urban towns leaving the rural areas with little or no dental services. Data from unpublished sources revealed that the level of dental education in the population is low and self-medication for oral health problems is a common practice.

Several studies have documented the oral microbiota in diabetic patients as well as other patient populations (5, 13, 14). Sharma and co-workers (2010) reported that both Gram positive and Gram negative bacteria are fairly involved in dental diseases and that the prevalence of bacteria increases with severity of disease. In another related study, it was reported that periodontal pathogens were different in diabetic patients and non-diabetic controls as well as in aggressive and chronic periodontitis (15). Khovidhunkit and colleagues (2009) noted the predominant microbes isolated from saliva to be mutans streptococci, lactobacilli and *Candida* sp. Also, a high prevalence (48.0%) of Enterobacteriaceae in the oral cavities of the denture-wearing population compared with 16.4% in the normal population has been reported (16). Most of previous studies associating oral microbial flora with oral infections in diabetic patients were focused on anaerobic bacteria. The few studies on aerobic flora (17) in diabetes are focused on different anatomical sites other than the oral cavity and little is known of the role of aerobic oral flora in oral infections in diabetes patients. The aim of this study is to compare aerobic oral microbial flora in diabetic patients and non-diabetic controls and to relate these microbes with oral infections and oral hygiene.

MATERIALS AND METHODS

The study was a cross-sectional research involving 265 participants (154 diabetic patients and 111 non-diabetic controls). Participants aged 18 years and above were recruited either from diabetic clinics or from the general population. Diabetes was confirmed by fasting blood sugar levels ≥ 126mg/dl, the use of hypoglycemic drugs and a history of diabetes. (18). Written consent was obtained from all patients. The study protocol was approved by the Ethical Clearance Committee of the University of Buea, Cameroon. Participants were asked to complete questionnaires bearing information on their demographic and medical history. Three millilitres of blood and oral washes were collected from each participant. The blood was used to test for plasma blood sugar as previously reported (18). To obtain the oral washes, each subject was asked to rinse his/her mouth with about 10ml of sterile Phosphate Buffered Saline (PBS) for 1 minute. The suspension was dispensed into a sterile collection cup (Equator Medical Inc., UK), placed in a cool box and transported to the laboratory within 2 hours of collection. The concentrated oral rinse technique was employed to culture both bacteria and fungi and procedures were conducted as previously described (19). Each oral rinse was transferred under aseptic conditions into a 15ml falcon tube, centrifuged at 2500rpm for 5 minutes and pellet was re-suspended in 1ml of PBS. Ten microlitres of the suspension was used to inoculate the following culture media: Blood agar supplemented with Colimycin-Nalidixic (CNA) acid mixture, this medium is selective for Gram positive bacteria; Chocolate agar supplemented with Polyvitex and Vancomycin-Colimycin-Nystatine mixture, MacConkey agar and Sabouraud's agar (BioMerieux SA., France) for the isolation of fungi. Culture plates were incubated at 37^{o}C under aerobic conditions for 24-48 hours, after which plates were observed for microbial growth. Then plates were examined for significant bacteria growth and characteristic colonial morphology. The number of colonies on each plate was counted and the number of colony-forming units (CFU) per ml calculated to indicate microbial density. For each characteristic morphotype, 5 distinct colonies were randomly selected and sub-cultured on appropriate medium, for identification. Isolates were Gram stained as previously reported (20) and then identified by

standard biochemical tests. The Germ tube test was used to distinguish *Candida albicans* from other *Candida* species (11). To assess oral infections, oral exam was performed by a dentist based on World Health Organisation standards (21) and participants were classified as having one of the following: dental caries, gingivitis or periodontitis. Oral health status was analysed using the simplified oral hygiene index OHI-S (22) and participants were grouped into Good, Fair and Poor oral hygiene. Data from this study were expressed as frequencies and statistical significances were assessed using the Chi-square test. All data were analysed using SPSS (version 17.0) at 95 % confidence level and P-values < 0.05 were considered statistically significant.

RESULTS

A. Distribution of Oral Isolates among Study Participants

Most of the study participants were in the age group 50 years and above and were more females (150, 56.6 %) in the study than males (115, 43.4 %). Of the 265 participants, 264 (99.62%) had at least one of the isolates. **Table 1** presents the prevalence of microbes from the oral cavity of study participants. Isolates belonging to 13 genera were identified including; *Candida, Klebsiella, Serratia, Stapylococcus, Streptococcus, Escherichia, Acinetobacter, Providencia, Flavimonas, Burkholderia, Kluyvera, Citrobacter* and *Enterobacter*. Generally, the most prevalent microbes amongst study participants were viridans *Streptococcus* (163, 99.6 %), *Candida albicans* (45, 17.0 %), *Serratia* Spp (19, 7.2 %), other *Candida* Spp (18, 6.8 %), CNS (17, 6.4 %) and *Klebsiella* Spp (15, 5.7 %). Gram positive bacteria (GPC) were the most frequently isolated (281, 68.2 %) from the oral cavity; followed by Gram negative bacteria (GNR) (68, 16.5 %), then yeasts (63, 15.3 %).

TABLE 1: DISTRIBUTION OF ORAL ISOLATES IN DIABETICS AND NON-DIABETICS

Mouth Isolates	Diabetics n (%)	Non-diabetics n (%)	Total n (%)
Candida albicans	30 (19.5)	15 (13.6)	45 (17.0)
Citrobacter Spp	1 (0.6)	0 (0.0)	4 (0.4)
GBS	1 (0.6)	0 (0.0)	1 (0.4)
Viridans Streptococcus	153 (99.4)	110 (100.0)	263 (99.6)
Acinetobacter Spp	0 (0.0)	1 (0.9)	1 (0.4)
Burkholderia	2 (1.3)	0 (0.0)	2 (0.8)
Other *Candida* Spp	12 (7.8)	6 (5.5)	18 (6.8)
E. coli	1 (0.6)	0 (0.0)	1 (0.4)
Enterobacter Spp	2 (0.1.3)	0 (0.0)	2 (0.8)
Flavimonas Spp	0 (0.0)	1 (0.9)	1 (0.4)
GNR*	8 (5.2)	16 (14.5)	24 (9.1)
Klebsiella Spp	8 (5.2)	7 (6.4)	15 (5.7)
CNS	14 (9.1)	3 (2.7)	17 (6.4)
Providencia Spp	0 (0.0)	1 (0.9)	1 (0.4)
Serratia Spp	10 (6.5)	9 (8.2)	19 (7.2)
Kluyvera Spp	1 (0.6)	0 (0.0)	1 (0.4)
Total **	154 (58.3)	110 (41.7)	264 (100)

*** GNR represents gram-negative rods which were isolated but unable to type by available biochemical tests.**
****Percentage based on the number of respondents**

B. Comparison of Oral Isolates between Diabetics and Non-Diabetics

Figure 1 demonstrates the distribution of oral microbes among diabetics and non-diabetics. There was a significant difference in the rate of isolation of yeasts (*Candida* spp) from the oral cavity between diabetics and non-diabetics (**χ2-test: P < 0.001**). Yeasts were frequently isolated from the oral cavity of diabetic patients (42, 66.7 %) than non-diabetics (21, 33.3 %). Also, more Gram positive bacteria were isolated from diabetics than non-diabetics (**168, 59.8** % versus **113, 40.2** %) but this difference was not statistically significant. Similarly, the distribution of Gram negative bacteria among diabetics and non-diabetics did not show any significant difference (**χ2-test: P = 0.732**).

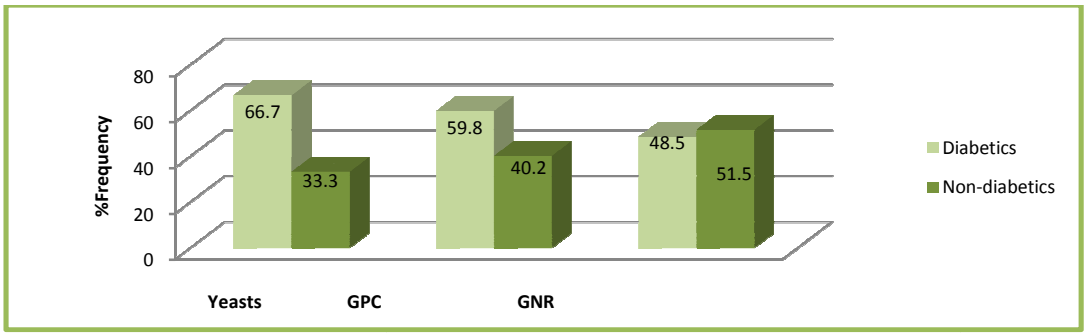

FIGURE 1: FREQUENCY OF ISOLATION OF ORAL MICROBES IN DIABETICS AND NON-DIABETICS

TABLE 2: DISTRIBUTION OF ORAL ISOLATES WITH RESPECT TO ORAL HYGIENE STATUS OF STUDY PARTICIPANTS

Oral Isolates	Oral hygiene status			Chi Square Test
	Good n (%)	Fair n (%)	Poor n (%)	
Candida spp	17 (19.3)	30 (24.6)	14 (42.4)	$\chi 2 = 6.849$; P = 0.033
Gram positive bacteria	88(100)	122 (100)	33 (100)	-
Gram negative bacteria	21 (23.9)	32 (26.2)	10 (30.3)	$\chi 2 = 0.530$; P = 0.767
Total	88	122	33	

C. Distribution of Microbes with respect to Oral Infections in Diabetes Patients

Table 3 shows the isolation of microbes with respect to oral infections in diabetics. Thirty eight diabetics were diagnosed with gingivitis, 39 with periodontitis and 45 with dental caries. Gram positive bacteria were isolated from all cases of oral infection. Gram negative bacteria were significantly isolated from cases of dental caries (**$\chi 2$ Test: P= 0.021**). Although more *Candida* sp was isolated from cases of dental caries, the distribution of *Candida* with respect to oral disease was not significant (**$\chi 2$ Test: P > 0.05**).

TABLE 3: DISTRIBUTION OF ORAL ISOLATES WITH RESPECT TO ORAL DISEASE IN DIABETES PATIENTS

Oral Isolates	Oral disease					
	Gingivitis		Periodontitis		Dental caries	
	n (%)	$\chi 2$ Test	n (%)	$\chi 2$ Test	n (%)	$\chi 2$ Test
Candida spp	11(28.9)	P=0.75	14 (35.9)	P=0.393	7 (37.8)	P=0.087
Gram positive bacteria	38 (100)	-	39 (100)	-	45(100)	-
Gram negative bacteria	12 (31.6)	P= 0.17	12 (30.8)	P = 0.61	14(31.1)	P=0.02
Total	38		39		45	

DISCUSSION

In the present study, the distribution of microorganisms in the oral cavity was analysed and the prevalence was compared between diabetics and non-diabetics in order to determine the role of diabetes on oral microbial colonization by aerobic microbes. The most commonly isolated microbes from the oral cavity of participants were *Streptococcus* sp (263, 99.6%), *Candida albicans* (45, 17.0%), *Serratia* (19, 7.2%), other *Candida* sp (18, 6.8%), CNS (17, 6.4%) and *Klebsiella* (15, 5.7%), (**Table 1**). These results are concurrent with reports from other studies which showed that streptococci, staphylococci and *Serratia* were among the

predominant oral isolates in both diabetics and non-diabetics (13). Mutans Streptococcus, Lactobacilli and *Candida* spp have been identified as the predominant microbes from saliva of diabetes patients (14). Oral Gram negative aerobic bacteria have been shown to play a role in infections in diabetic patients (23). Isolation of oral isolates was compared between diabetics and non-diabetics (**Figure 1**). It was revealed that yeasts (*Candida*) were significantly isolated from diabetics than non-diabetics ($P<0.001$). Yeasts were isolated from 66.7 % of diabetic patients and 33.3 % of non-diabetic controls. However, no significant difference in the isolation of aerobic bacteria was observed in the two groups. These results corroborated with that of other studies (11, 24). Abu-Elteen and co-workers (2006) reported oral Candida in 58.3% of diabetics compared with 30% in healthy controls. The high prevalence of oral candidal colonization has been attributed to the effect of hyperglycaemia (11). Results from this study revealed that diabetes mellitus might enhance oral candidal colonization and proliferation (11, 25). On the contrary, Pinducciu and colleagues (1997) did not notice any significant difference in the isolation of both anaerobic and aerobic microbial flora in 31 diabetics and 20 non-diabetics. This difference might be due to discrepancy in the number of subject as our study was based on a higher study population (154 diabetics and 111 non-diabetics).

Recently, socioeconomic status has been associated with poor oral hygiene and consequently, oral infections. In the present study, oral hygiene was significantly associated with microbial (yeast) colonization (**Table 2**). In a recent study, it was noted that diabetes was an added risk for oral disease in the low-income population of Northern Manhattan (12). The authors suggested that oral disease prevention and treatment programs may need to be part of the standards of continuing care for patients with diabetes. Apart from hyperglycemia and smoking, denture-wearing has also been reported to influence oral candidal colonization (24). The mechanism for this is not fully understood but it is probably due to the effect of denture wearing on oral hygiene. The association of microbes and oral infections is increasingly being reported (27, 28). Sharma and colleagues (2011) reported both gram positive and gram negative organisms to be fairly involved in dental diseases in diabetic patients. In another related study, streptococci and enteric bacteria were frequently isolated from dental root canal in clinically asymptomatic cases of periapical pathosis (29). In the present study, we noticed a significant association between Gram negative aerobic bacteria and dental caries (**Table 3**). Our result is in line with that of Sharma and co-workers (2011) in which both aerobic and anaerobic gram negative bacteria were associated with periodontitis, dental caries and gingivitis.

From the present study, it can be concluded that diabetics and non-diabetics may harbour different oral microorganisms which may alter their oral health and that aerobic Gram negative bacteria may play an important role in dental diseases in diabetic patients.

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Author's Contributions

MEAB – Conception, sample collection and analysis, data analysis and compilation of results

PNF – Verification of data and results, supervision and guidance

KFHL – Substantial review of the manuscript for final publication

TNA – Conception, verification of data and results, supervision and guidance

REFERENCES

1. Aas J. A., Paster B. J., Stokes L. N., Olsen I., Dewhirst F. E. Defining the normal bacterial flora of the oral cavity. J. Clin. Microbiol. 2005; 4: 5721-5732.
2. Bagg J. Can the colonisation resistance of the oral microflora be reduced? Microb. Ecol. Health Dis. 1990; 3: v-viii.
3. Demmer, Ryan T., Desvarieux, Moise. Periodontal infections and cardiovascular disease: The heart of the matter. J. Am. Dental Assoc. 2006; 137: 14-20.
4. Li X., Kolltveit K. M., Tronstad L., Olsen, I. Systemic diseases caused by oral infection. Clin. Microbiol. Rev. 2000; 13: 547-558.
5. Ewan V., Perry J. D., Mawson T., McCracken G., Brown A. N., Newton J., Walls A. Detecting potential respiratory pathogens in the mouths of older people in hospital. Age Ageing. 2010; 39: 122-125.
6. Boggess K. A., Edelstein, B. L. Oral health in women during preconception and pregnancy: implications for birth outcomes and infant oral health. Matern. Child Health J. 2006; 10: 169-174.
7. Fernandes J. K., Wiegand R. E., Salinas C. F., et al. Periodontal disease status in Gullah African Americans with type 2 diabetes living in South Carolina. J. Periodont. 2009; 80: 1062–1068.

8. Orbak R., Simsek S., Orbak Z., Kavrut F., Colak M. The Influence of Type-1 diabetes mellitus on dentition and oral health in children and adolescents. Yonsei Med. J. 2008; 49(3): 357–365.
9. Sarnat H., Eliaz R., Feiman G., Flexer Z., Karp M., Laron Z. Carbohydrate consumption and oral status of diabetic and non-diabetic young adolescents. Clinical Prev. Dent. 1985; 7: 20-23.
10. Siudikienė J., Mačiulskienė V., Dobrovolskienė R., Nedzelskienė I. Oral hygiene in children with type I diabetes mellitus. Stomatologija, Baltic Dent. Maxillofacial J. 2005; 7:24-7.
11. Ghannoum M. A., Jurevic R. J., Mukherjee P. K., et al. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathogens. 2010; 6: e1000713.
12. Lalla E., Park D. B., Papapanou P. N., Lamster I. R. Oral disease burden in Northern Manhattan patients With diabetes mellitus. Am. J. Public Health. 2004; 94: 755–758.
13. Sharma M., Tiwari S.C., Singh K., Kishor K. Occurrence of bacterial flora in oral infections of diabetic and non-diabetic patients. Life Sci. Med. Res. 2011; 32.
14. Khovidhunkit S. P., Suwantuntula T., Thaweboon S., Mitrirattanakul S., Chomkhakhai U., Khovidhunkit W. Xerostomia, Hyposalivation, and Oral Microbiota in Type 2 Diabetic Patients: A Preliminary Study. J. Med. Assoc. Thailand. 2009; 92: 1220-1228.
15. Yacoubi A. Microbiology of periodontitis in diabetic patients in Oran, Algeria. Ibnosina J. Med. BS. 2013; 5: 280-287.
16. Goldberg S., Cardash H., Browning H., Sahly H., Rosenberg M. Isolation of Enterobacteriaceae from the mouth and potential association with malodor. J. Dent. Res. 1997; 76: 1770-1775.
17. Arbab T. M., Qadeer S., Iqbal S., Mirza M.A. Aerobic bacterial conjunctival flora in diabetic patients. Pak. J. Ophthalmol. 2010; 26: 177-181.
18. ADA. American Diabetes Association report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 1997; 20: 1183–1197.
19. Coulter W. A., Murray S. D., Kinirons M. J. The use of a concentrated oral rinse culture technique to sample oral candida and lactobacilli in children and the relationship between candida and lactobacilli levels and dental caries experience: a pilot study. Intern. J. Paediatr. Dentist. 1993; 3: 17-21.
20. Cheesbrough, M. District Laboratory Practice in Tropical Countries Part 2. Second Edition. Cambridge University Press 2006.
21. World Health Organization. Oral health Survey: Basic methods. Geneva: World Health Organization. 1997.
22. Greene J. C., Vermillion J. R. The simplified oral hygiene index. J Am Diet Assoc. 1964; 68: 7-13.
23. Mackowiak P. A., Martin R. M., Smith J. W. The role of bacterial interference in the increased prevalence of oropharyngeal gram-negative bacilli among alcoholics and diabetics. Am. Rev. Resp. Dis. 1979; 120: 589-593.
24. Abu-Elteen K. H., Hamad M. A., Salah S. A. Prevalence of oral Candida infections in diabetic patients. Bahrain Med. Bulletin. 2006; 28: 1-8.
25. Aly F. Z., Blackwell C. C., Mackenzie D.A.C., Weir D. M., Clarke B. F. Factors influencing oral carriage of yeast among individuals with diabetes mellitus. Epidemiol Infect.1992; 109: 507-518.
26. Pinducciu G., Micheletti L., Piras V., et al. Periodontal disease, oral microbial flora and salivary antibacterial factors in diabetes mellitus type 1 patients. Eur. J. Epidem. 1997; 12: 631-636.
27. Boutaga K., Winkelhoff A. J. V., Vandenbroucke-Grauls C. M. J. E., Savelkoul P. H. M. Comparison of Real-Time PCR and culture for detection of Porphyromonas gingivalis in subgingival plaque samples. J. Clin. Microbiol. 2003; 41: 4950-4954.
28. Gafan G. P., Lucas V. S., Roberts G. J., Petrie A., Wilson M., Spratt D. A. Prevalence of periodontal pathogens in dental plaque of children. J. Clin. Microbiol. 2004; 42: 4141–4146.
29. Yoshida M., Fukushima H., Yamamoto K., Ogawa K., Toda T., Sagawa H. Correlation between clinical symptoms and microorganisms isolated from root canals of teeth with periapical pathosis. Am. Assoc. Endodontists. 1987; 13.

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PREVALENCE OF TUBERCULOSIS AMONGST PATIENTS ATTENDING TWO SECONDARY HOSPITALS IN ABEOKUTA OGUN STATE.

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ABSTRACT

This study was conducted to examine the rate of *Mycobacterium tuberculosis* infection among individuals attending the outpatient clinic of two hospitals in Abeokuta Metropolis in Southwestern Nigeria. Of the 132 individuals examined, the overall rate of tuberculosis infection was 16.7%. Infection was highest among patients in the 21-40 year age group (11.4%). Results also showed that 10.6% of male patients were infected with tuberculosis and 6.1% of female patients infected with tuberculosis. There was no significant difference between the sex and *Mycobacterium tuberculosis* infection. There was also no significant difference between age-groups and *Mycobacterium tuberculosis* infection. But there was a significant difference between the ESR and tuberculosis infection.

PREVALENCE DE TUBERCULOSE PARMI LES PATIENTS SUIVIS DANS DEUX HOPITAUX DE SOINS SECONDAIRES DE ABEOKUTA, ETAT DE OGUN

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Résumé

Cette étude a été menée afin d'examiner le taux d'infections à *Mycobacteriumtuberculosis*chez les individus suivis à la clinique externe de deux hôpitaux de métropole de Abeokuta au Sud-ouest du Nigéria. Sur les 132 individus examinés, le taux global d'infection de la tuberculose était de 16,7%. L'infection était plus élevée chez les patients âgés de 21-40 ans (11,4%). Les résultats ont également montré que 10,6% des patients de sexe masculin ont été infectés par la tuberculose et 6,1% des patientes infectées par la tuberculose. Il n'y avait pas de différence significative entre le sexe et l'infection à *Mycobacteriumtuberculosis*. Il n'y avait pas de différence significative entre les groupes d'âge et l'infection à *Mycobacteriumtuberculosis*. Mais il y avait une différence significative entre l'ESR et la tuberculose.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacterium Mycobacterium tuberculosis. It was first described in 1882 by Robert Koch, who won the Nobel Prize in Physiology/ Medicine for this discovery in

1905 (1). It essentially attacks the lungs in more than 80% of the cases, leading to primary tuberculosis.

Extra-pulmonary tuberculosis occurs in less than 20% of cases and affects various organs such as lymph

nodes, meninges, intestine, bone and joints among others(2).

Tuberculosis is an infectious disease causing mortality in humans. Timely detection of the disease permits

the institution of effective, life-saving treatment, and thereby reduces transmission to close contacts. Despite

the technical advancement in tuberculosis research, improved environmental conditions especially in the

developed countries and the discovery of effective treatment half a century ago, the disease remains a health

problem worldwide (3). *M. tuberculosis* infection remains the leading infectious killer of youth and adults.

Tuberculosis (TB) is the leading cause of death in the world from a bacterial infectious disease. The disease

affects 1.8 billion people per year which is equal to one-third of the entire world population (4).

Mycobacterium tuberculosis, along with *M. bovis*, *M. africanum* and *M. microti* all cause the disease known as

tuberculosis and are members of the Mycobacteria tuberculosis complex. Each member of the complex is

pathogenic, but *Mycobacterium tuberculosis* is for humans while *Mycobacterium bovis* is usually pathogenic for

animals (5). Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread

through droplet or aerosol (6).

M. tuberculosis is classified as an acid fast bacterium and it divides every 15- 20 hours, which is slow when

compared to other bacteria, which tend to have division times measured in minutes. It's a small, rod-like

bacillus that can withstand weak disinfectants and can survive in dry state for weeks (7). *Mycobacterium*

tuberculosis is identified microscopically by its staining characteristics; it retains stains after being treated

with acidic solution, and is thus classified as an acid fast bacillus (AFB). In Ziehl-Neelsen staining

procedure, AFB are stained a bright red, and can also be visualized by fluorescent microscopy and diagnosis

can be made using polymerase chain reaction (PCR) (8).

Most infections in humans result in an asymptomatic, latent infection and about one in ten latent infections

eventually progresses to active disease which if left untreated, kills more than 50% of its victims.

The classic symptoms are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss

(the last giving rise to the formerly prevalent colloquial term "consumption"). Infection of other organs

causes a wide range of symptoms (9). Diagnosis relies on radiology (commonly chest X-rays), a skin

test, blood tests, as well as microscopic examination and microbiological culture of bodily fluids. Treatment

is difficult and requires long courses of multiple antibiotics. Contacts are also screened and treated if

necessary (6). Antibiotic resistance is a growing problem in (extensively) multi-drug-resistant tuberculosis.

Prevention relies on screening programs and vaccination, usually with Bacillus Calmette-Guérin vaccine

(10).

Although, one third of the world's population is thought to be infected with *M. tuberculosis* (11), new

infections occur at a rate of about one per second (12). The proportion of people who become sick with

tuberculosis each year is stable or falling worldwide but, because of population growth, the absolute

number of new cases is still increasing (12). In 2007 there were an estimated 13.7 million chronic active cases, 9.3 million new cases, and 1.8 million deaths, mostly in developing countries (13). In addition, more people in the developed world are contracting tuberculosis because their immune systems are compromised by immunosuppressive drugs, substance abuse, or AIDS. The distribution of tuberculosis is not uniform across the globe; about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5-10% of the US population test positive (14).In another study conducted in umuahia by Nwachukwu et al it was reported that the overall prevalence of *M. tuberculosis* infections was 21.6% (15). The objective of this study is to determine the prevalence of tuberculosis among patients using two major hospitals in Abeokuta, Ogun State.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Abeokuta, the capital city of Ogun State, Southwest Nigeria. The hospitals selected for this study were Sacred Heart Hospital, Lantoro and State General Hospital, Ijaiye, Abeokuta. The hospitals were selected because they are reference centers for the diagnosis and treatment of tuberculosis (TB).

SAMPLE COLLECTION

The samples were collected randomly among suspected TB patients attending the outpatient clinic at Sacred Heart Hospital, Lantoro and State General Hospital, Ijaiye, Abeokuta within the period of March to April, 2011. The samples collected were blood and sputum.

Sputum Collection

One hundred and thirty two sputum samples were collected for this study. Three sputum specimens were collected from each patient. These were 'first spot' specimen, an early morning specimen and a 'second spot' specimen. The selected patients were given two dry clean, universal containers each. They were instructed to produce sputum from a deep cough into one of the containers on the first day of visitation to the clinic (first spot specimen). The patients took the second container home and were instructed to produce early morning sputum from a deep cough before food (early morning specimen). On arrival to the laboratory with the early morning specimen, another sputum specimen (second spot specimen) was collected from each patient.

MICROSCOPIC EXAMINATION OF THE SPUTUM

Equal volume of sputum specimen and 4% sodium hydroxide were mixed together, centrifuged at 3000 rev/ min for 15 minutes and a wooden applicator was used to transfer an appropriate part of the sediment onto the slide and smeared in a repeated coil type on the middle of a clean microscope slide over an area approximately 2cm by 1cm. The smear was allowed to dry on the bench for 15 minutes and it was fixed by passing through flame 3-4 times with the smear uppermost. The fixed smear was flooded with Ziehl-Neelsen Carbol Fuchsin and slide was heated slowly until it was steamed. Steaming was maintained for 3-5 minutes by using low or intermittent heat. The slide was rinsed in a gentle stream of running water until the excess stain is washed away. The slide was flooded with decolorizing solution (3% acid-alcohol) and it was left for 3 minutes. The slide was rinsed thoroughly with water and excess was drained. The slide was flooded with methylene blue to counter stain and was left for 1 minute. The slide was rinsed thoroughly with water and excess water was drained. The slide was allowed to air dry and was viewed under the oil immersion microscope. The results of sputum examination can either be positive or negative.

Positive results include: 1+, 2+ and 3+

The explanation of indices is as follows;

TABLE 1: INTERPRETATION OF RESULTS

(0): means no AFB is seen in at least 100 fields, therefore it's reported as negative.

(1+): means 10-99 AFB are seen in 100 fields.

(2+): means 1-10 AFB per field are seen in at least 500 fields.

(3+): means more than 10 AFB per field are seen in at least 20 fields

The Erythrocyte Sedimentation Rate (ESR) of each patient was determined by Westergen-micro method using EDTA anticoagulated blood and the reading was taken at the end of one hour. The normal value for ESR is 0-10 mm/hr.

RESULTS

A total number of one hundred and thirty two (132) individuals attending outpatient clinic at Sacred Heart Hospital and State General Hospital, Ijaiye in Abeokuta participated in this study. Out of the total number 72(54.5%) were males while 60(45.5%) were females.

The highest number of patients were in the age group 21-40 years, 64(48.5%) and the least in the age group 61-81 years, 10(7.6%). The prevalence of *Mycobacterium tuberculosis* infection in relation to age is shown table 2. The most infected individuals were seen in the age group 21-40 years, 15(11.4%) while the least infected individuals were seen in the age group 61-81 years, 1(8.0%). Age did not contribute significantly to mycobacteria infection. (P= 6.752)

TABLE 2: *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN RELATION TO AGE

Age (year)	Total number examined (%)	*Mycobacterium tuberculosis* infection	
		Positive (%)	Negative (%)
0-20	18 (13.6)	4 (3.0)	14 (10.6)
21-40	64 (48.5)	15 (11.4)	49 (37.1)
41-60	40 (30.3)	2 (1.5)	38 (28.8)
61-81	10 (7.6)	1 (8.0)	9 (6.8)
Total	132 (100.0)	22 (16.7)	110 (83.3)

X^2= 6.752 P= 0.080

Table 3, Showed that the overall prevalence of *M. tuberculosis* was 22(16.7%) of which 14(10.6%) were males and 8(6.1%) were females. Sex did not contribute significantly to *M. tuberculosis* infection (P= 0.880)

Table 5 Showed the results of the cross-tabulation between M.tuberculosis infection and Erythrocyte Sedimentation Rate (ESR). It showed that none of the patient with *M. tuberculosis* infection had normal Erythrocyte Sedimentation Rate (2-10mm/hr). ESR is significantly affected by mycobacterium tuberculosis (P=0.001)

TABLE 3: *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN RELATION TO SEX

Sex	Total number examined (%)	*Mycobacterium tuberculosis* infection	
		Positive (%)	Negative (%)
Male	72 (54.5)	14 (10.6)	58 (43.9)
Female	60 (45.5)	8 (6.1)	52 (39.4)
Total	132 (100.0)	22 (16.7)	110 (83.3)

X^2= 0.880 P= 0.348

TABLE 4: *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN RELATION TO PCV (PACKED CELL VOLUME)

PCV (Range)	Total number examined (%)	*Mycobacterium tuberculosis* infection	
		Positive (%)	Negative (%)
Normal (35-54)	94 (71.2)	0 (0.0)	94 (71.2)
Moderate (26-34)	21 (15.9)	5 (3.8)	16 (12.1)
Severe (< 26)	17 (12.9)	17 (12.9)	0 (0.0)
Total	132 (100.0)	22 (16.7)	110 (83.3)

X^2= 95.895 P= 0.001

TABLE 5: *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN RELATION TO ESR (ERYTHROCYTE SEDIMENTATION RATE)

ESR	Total number examined (%)	*Mycobacterium tuberculosis* infection	
		Positive (%)	Negative (%)
2-10	107 (81.1)	0 (0.0)	107 (81.1)
11-40	5 (3.8)	2 (1.5)	3 (2.3)
41-70	9 (6.8)	9 (6.8)	0 (0.0)
71-100	11 (8.3)	11 (8.3)	0 (0.0)
Total	132 (100)	22 (16.7)	110 (83.3)

X^2= 112.218 P= 0.001

DISCUSSION

This study showed an overall prevalence of 16.7% of Mycobacterium tuberculosis infection among patients in Abeokuta, Ogun State, South–Western Nigeria. In another study in Abeokuta, South-western Nigeria[3] a lower prevalence of *M. tuberculosis* among HIV pregnant women attending antenatal clinics and Onifade et al in Lagos, South-west, Nigeria also recorded a lower prevalence of *M. tuberculosis* infection although they employed mantoux test for screening.(16).

In Maiduguri, Northern Nigeria, Ukwandu et al reported higher prevalence of *M. tuberculosis* infection (17). In Minna, Ibrahim *et al.* also

obtained a higher prevalence of *M. tuberculosis* and Okodua *et al.* obtained higher prevalence for TB-HIV co-infection in Edo State, Nigeria (18,19). Other states in Nigeria like Kano, Enugu, Borno, Plateau and Benue recorded prevalent rates of 12.0, 14.0, 27.0, 30.0 and 35.0% respectively (20).

There was a higher prevalence rate in males (10.6%) when compared to females (6.1%). This higher rate could be due to higher exposure to HIV infection which inadvertently predisposed more of the affected males to TB disease. Though results of this study show that there was no significant difference between the sex and *M. tuberculosis* infection. This supports the report of Nwachukwu et al[15]. Although the highest rate of infection was among the age group 21-40 years, there was no significant difference between age group and *M. tuberculosis* infection for this study. This is in agreement with the previous report of Lawn et al (21). The lowest rate of infection in age group 61-81 may be due to diminished social habits. In the report of Campbell *et al.* which is similar to this study, *M. tuberculosis* infection significantly caused extreme increase in erythrocyte sedimentation rate (ESR). Studies also carried out by Ojo *et al.* supports this finding 3).

This study has been able to determine sputum smear positivity amongst patients who attended tuberculosis diagnosis and treatment clinics in Abeokuta. The infection was observed to affect mostly the economically supportive group of the society. The outcome of this project revealed that the prevalence of tuberculosis has been on decreasing side and the previous report of Ojo *et al.*[3] lend credence to this study.

CONCLUSION

In conclusion more enlightenment campaigns should be carried out in the state to help curtail the infection. The control of tuberculosis therefore should involve the government at all levels.

RECOMMENDATION

We recommend that improved diagnostic facilities be deployed to various tertiary and secondary health facilities for rapid diagnosis of TB and also susceptibility testing for Multidrug resistant tuberculosis.

International collaborations with low resource countries where TB is rampant should be established so that personnel can be trained to diagnose and treat TB appropriately.

REFERENCES

1.Soren, T., Markos, A., Pernille and Nyagosya, R. (2007). Clinical infectious disease. *Infectious Diseases Society of America*. **45**: 575-579.

2. Obionu, C. N. (2007). Primary Health care for developing countries (2nd ed.) Delta Publication (Nig) Ltd, Enugu. Pp. 139-140.

3. Ojo, D. A., Mafiana, C. F. and Adeniran-Sonola, A. (2007). Prevalence of *Mycobacterium tuberculosis* and human immunodeficiency virus infections. *Nigerian Journal of Parasitology*. Vol. (28): 39-40.

4. Todar, K. (2011). *Mycobacterium tuberculosis* and Tuberculosis. *Division of Tuberculosis Elimination.* Pp 1-4.

5. Crowle, A. J., Dahl, R., Ross, E. and May, M. H. (1999). Evidence that vesicles containing living, virulent *Mycobacterium tuberculosis* or *Mycobacterium avium* in cultured human macrophages are not acidic. *Journal of infection and immunity*. 59: 1823-1831.

6. Konstantinos, A. (2010). "Testing for tuberculosis". *Australian Prescriber* **33** (1): 12–18.

7. Ryan, K. J. and Ray, C. G. (2004). Sherris Medical Microbiology, 4th edition. Mc Graw. ISBN 0-838-8529-9.

8. Camus, J. C., Pryor, M. J., Medigue, C. and Cole, S. T. (2002). Re-annotation of the genome sequence of *Mycobacterium tuberculosis*. Microbiology. H37 Rv 2002: 2967-2973.

9. World Health Organization (WHO) WHO report 2008: Global tuberculosis control

10 Bonah, C. (2005). "The 'experimental stable' of the BCG vaccine: safety, efficacy, proof, and standards, 1921–1933". *Study of Histology*

Philosophy Biology Biomedical Science **36** (4): 696– 18. 721.

11. World Health Organization. (2010). WHO Tuberculosis Factsheet, pp. 105-120.

12. World Health Organization. (2007). Tuberculosis". Fact sheet Nº 104.

13. World Health Organization (2009). Epidemiology. *Global tuberculosis control: epidemiology, strategy, financing*. pp. 6–33.

14. Kumar, V., Abbas, A. K., Fausto, N. & Mitchell, R.N. (2007). *Robbins Basic Pathology* (8th ed.). Saunders Elsevier. pp. 516–522.

15. Nwachukwu, E. and Peter, G. A. (2010). Prevalence of *Mycobacterium tuberculosis* and human immuno deficiency virus (HIV) infections in Umuahia, Abia state, Nigeria. *African Journal of Microbiology Research* Vol. 4 (14) pp. 1486-1490.

16 Onifade, E. U. and Dasekum, E. O. (2002). Tuberculin test (Mantoux) reactions in an adolescent population in Lagos. *Nigerian Journal of Paediatrics*. **27**: 11-18.

17. Ukwandu, N. C. D. (1998). Evaluation of the Laboratory techniques used in the diagnosis of sputum- producing patients suspected of mycobacterium infection. *West African Journal of Medicine*. **17**: 38-41.

18. Ibrahim K., Akanni, O.O. and Ijah, U. J. (2004). The prevalence of tuberculosis in HIV patients in Minna metropolis. *Nigerian Journal of Microbiology*. **18**: 212-216.

19. Okodua, M. A., Nwobu, G. O., Tatfeng, Y. M., Ongey, J. Y. and Agwu, E. (2004). Incidence of HIV – related pulmonary tuberculosis in Edo State, Nigeria. *Shiraz E-Medical. Journal*. **5** (1): 8-12.

20. Federal Ministry of Health (2000). Tuberculosis and Leprosy control efforts in Nigeria. National Tuberculosis and Leprosy control programme. (NTBLCP). Pp. 150.

21. Lawn, S. D. and Achaempong, J. W. (1999). Pulmonary Tuberculosis in adults: factors associated with mortality at a Ghanaian teaching hospital. *West African Journal of Medicine*. **18**: 270-274.

22. Campbell, C. C., Zucker, J. R., Lackritz, E. M., Ruebush, T. K., Hightower, A. W., Adingoci, J. C. and Were, B. (1994). Anaemia, blood transfusion practices, HIV and mortality among women of reproductive age in Western Kenya. *Transaction of the Royal Society. Tropical. Medical Hygiene*. **88**: 172-176.

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SEROPREVALENCE SURVEY OF RUBELLA ANTIBODIES AMONG PREGNANT WOMEN IN MAIDUGURI, BORNO STATE, NIGERIA

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ABSTRACT

Rubella is a vaccine- preventable viral infection. Its etiologic agent, rubella virus was identified as a human teratogen capable of causing spectrum of birth defects described as congenital rubella syndrome (CRS) if the pregnant mother is infected within the first trimester of pregnancy. A total of 90 pregnant women attending a secondary health care facility in Maiduguri were screened for IgM and IgG antibodies using enzyme linked immunosorbent assay (ELISA) kit (Cortez Diagnostics Inc. USA). Of these, 37.8% (34/90) and 83.3% (75/90) were seropositive for anti-rubella IgM (x^2 Cal 5.1; p=0.05) and IgG respectively. Chi-square analysis (x^2 Cal 38.38, p=0.05// x^2 tab 31.41, p=0.05) revealed an association between miscarriage and IgG antibody level in twenty-one subjects. Pregnant women within 20-24years had the highest prevalence of 40% (36/90)(x^2 Cal 4.22, p=0.05) : 44.4% (16/36) of them were seropositive for IgM (x^2 Cal 4.31, p=0.05). A marked surge in IgG antibody level, which tantamount acute infection, was observed in 15.6% (14/90)(x^2 Cal 19.85, p=0.05) of the pregnant women. Pregnant women in the first trimester seropositive for anti-rubella IgM were 36.4% (4/11), inferring that the fetuses of these women are susceptible to sequelae of rubella. This result highlights the consequence of rubella infection and confirms continuous circulation of rubella virus in the study area. There is need for vaccination of vulnerable population in order to ensure the control /elimination of rubella virus in Nigeria.

Key words: Rubella virus, teratogen, antibodies, Maiduguri

SURVEILLANCE DE SEROPREVALENCE DES ANTICORPS ANTI-RUBEOLE CHEZ LES FEMMES ENCEINTES A MAIDUGURI, ETAT DU BORNOU, NIGERIA

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RESUME

La rubéole est une infection virale évitable par la vaccination. Son agent étiologique, virus de la rubéole a été identifié comme un tératogène humain capable de provoquer le spectre de malformation congénitale décrite comme le syndrome de rubéole congénitale (SRC) si la femme enceinte est infectée au cours du premier trimestre de la grossesse. Au total, 90 femmes enceintes fréquentant un établissement de soins de santé secondaires à Maiduguri ont été dépistées pour le dosage des anticorps IgM et IgG à l'aide de kit immunoenzymatique (ELISA) (Cortez Diagnostics Inc. USA). Parmi elles, 37,8% (34/90) et 83,3% (75/90) étaient séropositives respectivement pour les anticorps anti-IgM (X^2 Cal. 5,1, *p=0,05*) et IgG de la rubéole. L'analyse Chi-carré (X^2 Cal. 38,38, *p=0,05* /X^2Tab. 31,41, *p=0,05*) a révélé une relation entre la fausse couche et le niveau d'anticorps IgG dans vingt-et-un sujets. Les femmes enceintes de 20 à 24 ans ont eu la plus forte prévalence de 40% (36/90) (X^2 Cal. 4,22, *p=0,05*): 44,4% (16/36) d'entre elles étaient séropositives pour les IgM (X^2 Cal. 4,31, *p=0,05*). Une augmentation remarquable de taux d'anticorps IgG,

équivalent à l'infection aiguë, a été observée chez 15,6% (14/90) (X^2 Cal. 19,85, *p=0,05*) de femmes enceintes.Les femmes enceintes au premier trimestre, séropositives aux IgM anti-rubéole, ont été de 36,4% (4/11), déduisant que les fœtus de ces femmes sont sensibles aux séquelles de la rubéole. Ce résultat souligne la conséquence de la rubéole et confirme la circulation continue du virus de la rubéole dans la zone d'étude. Il est nécessaire de vacciner la population vulnérable afin d'assurer le contrôle et/ou l'élimination du virus de la rubéole au Nigeria.

Mots clés: virus de la rubéole, tératogènes, anticorps, Maiduguri

INTRODUCTION

Infection with rubella virus during pregnancy, especially during the first trimester, can result in congenital rubella syndrome (CRS) (1). The burden of rubella infection in most developing countries is however not well documented because of lim-ited epidemiological data (2).

The symptoms of rubella infection include a rash, low-grade fever, arthralgia, and lymphadenopathy. In most cases, the disease is self-limiting and rarely causes complications (3). Complications of CRS may include miscarriage and severe abnormalities of the fetus, such as cataracts, retinopathy, heart defects, neurological deficits, and deafness (4).

No antiviral drugs are available for treating rubella or preventing transmission to the fetus. Vaccination programs are regarded as an effective tool to eliminate rubella and congenital rubella (5).

WHO estimates that worldwide more than 100,000 children are born with CRS each year, most of them in developing countries (6).

In some African Countries, 80% of children have been found to be positive for rubella antibodies by the age of 10 years (7). Post-epidemic rubella antibody prevalence in Ghana has been found to be 92% among pregnant women, with susceptibility associated with a younger age. In Eritrea, the prevalence of antibodies to rubella has been reported to be as high as 99% in some female population (3).

In Nigeria, past studies have revealed 14.3%, 3.9%, 10%, 16.3%, prevalence of anti-rubella IgM in Abuja, Benue, Benin and Ilorin respectively (2, 8, 9, 10) while 53%, 62.7%, 68.5%, 54.1% prevalence of anti-rubella IgG in Benin, Zaria, Ibadan, and Maiduguri respectively (9, 11, 12, 13) have been reported.

Till date there is no national program to vaccinate children and women against rubella (14). This indicates that rubella immunization is not included in Nigeria immunization schedule and thus infections during pregnancy may still occur.

The possibility of occurrence of rubella in Nigeria is corroborated by report of "a Nigerian woman who arrived in the United States in early March 2012 in approximately week 32 of pregnancy. In the United States, her pregnancy was complicated with oligohydramnios and severe growth retardation. She did not recall having had a rash illness during her pregnancy. Maternal serum collected 3 days after she had given birth tested negative at CDC for rubella IgM and positive for rubella IgG with a high avidity index. In March 2012, she was delivered of an infant in Alabama by cesarean delivery at 33 weeks' gestational age. At birth, the infant had generalized hemorrhagic purpura (a blueberry muffin rash) over the entire body, patent ductus arteriosus, cardiomegaly, thrombocytopenia, pneumonitis, anemia, and liver dysfunction. Approximately 1 month later, the infant was transferred to a pediatric hospital, where the infant died in April 2012. Cause of death was recorded as CRS (1).

No serological evaluation of combined IgG and IgM antibodies for previous and current rubella infections has been reported in a single study in the study area.

Therefore this study was carried out to determine the sero-prevalence of maternal anti-rubella IgG and IgM in pregnant women attending a secondary health facility in Maiduguri, Nigeria and to generate baseline data which can serve to prompt relevant health authority to appreciate the need to formulate feasible, implementable policy to mitigate the sequelae of rubella virus infection, not only in the study area but the country at large.

MATERIALS AND METHODS

Study area

This research was carried out in Fatima Ali Sheriff health center, Maiduguri, Borno State, Nigeria. The research spanned January – November 2013. Maiduguri is the capital of Borno State located in the north eastern region of Nigeria. The indigenes are predominantly Kanuri by tribe.

Ethical Clearance

Ethical approval for the study was obtained from the Ethical Board of the hospital. Informed oral consent was obtained from all subjects recruited into the study.

Study design

To allow for fair representation of sample, a cross-sectional, hospital –based design was utilized. Out of the one hundred and twenty antenatal attendees intended for the study, only ninety (75%) whose consent was sought and obtained participated in the research. A structured questionnaire was administered on each pregnant woman to obtain necessary demographic data which included age, trimester of pregnancy, history of measles, mumps, rubella (MMR) vaccination and experience of skin rash.

Exclusion criteria

Women who are not pregnant were excluded from the research.

Inclusion criteria

Only pregnant women were included in the research.

Specimen collection

Three milliliter (3ml) of blood was aseptically collected by venipuncture and serum was obtained by centrifuging at 3000rpm for 5 minutes (Agbede *et al.*, 2011). Serum was kept frozen at -20^{0}C in the Department of Immunology, University of Maiduguri Teaching Hospital until analysis was done.

Specimen Analysis

The Enzyme linked immunosorbent assay technique was employed for both IgM and IgG antibodies. Abiding strictly by manufacturer's instruction (Diagnostic Automation, Inc. U.S.A), one in forty (1:40) dilutions of specimen, negative control, positive control and calibrator were prepared by adding 5µl of the aforementioned to 200µl of sample diluent and mixed well. One hundred microliter (100µl) of diluted sera, calibrator and controls were dispensed into the appropriate wells. One hundred microliter (100µl) of absorbent solution was dispensed in 1A well position for the reagent blank. The holder was tapped to remove air bubbles from the liquid and was mixed well and incubated for 30 minutes at room temperature. Liquids from all wells were removed and washed three times repeatedly with washing buffer. One hundred microliter (100µl) of enzyme conjugate was dispensed into each well and incubated for 30 minutes at room temperature. The enzyme conjugate was then removed from all wells and washed repeatedly three times with washing buffer. One hundred microliter (100µl) of TMB Chromogenic Substrate was dispensed to each well and incubated for 15 minutes at room temperature. Then 100µl of 2 N HCl was added to stop reaction. While ensuring there were no air bubbles in each well, O.D. at 450 nm was read with a microwell reader.

Interpretation of Result

Based on manufacturer's instruction (Diagnostic Automation, Inc. U.S.A), the mean value of Rubella G Index for each specimen was calculated by dividing the mean absorbance value of each sample by the cut off calibrator mean value. A sample was then considered positive for anti-Rubella IgG and IgM antibody whenever a Rubella G and M Index value is equal to or greater than 1.0, and considered negative whenever a Rubella G and M Index value is equal to or less than 0.90.

Criteria for the validity of the IgM assay

The assay was considered valid:

1. When the OD450nm of the A1 blank well was <0.150
2. When the OD450 nm of the calibrator was >0.250

Criteria for the validity of the IgG assay

The assay was considered valid:

1. When the OD450nm of the A1 blank well was <0.250

2. When the OD450 nm of the calibrator was >0.250

RESULTS

An overall prevalence of 37.8% and 83.3% for anti-rubella IgM and IgG antibodies respectively were obtained in this study (Table 1). Pregnant women within the age bracket 15-19years and 20-24years combined, constitute 58.88% of the respondents (Table 1) while majority, 62.22% to be precise, of the pregnant women sampled were in the third trimester of pregnancy. None of the respondent (0%) was 40years old (Table 1). Prevalence of pregnant women in the first trimester seropositive for rubella IgM was 36.4% (Table 1). There was evidence of marked surge in IgG antibody level among fourteen (15.6%) pregnant women (Table 2). Chi-square analysis (x^2 Cal 38.38, p=0.05// x^2 tab 31.41, p=0.05) revealed an association between miscarriage and IgG antibody level in twenty-one pregnant women (Table 1).

TABLE 1: DISTRIBUTION OF ANTI-RUBELLA ANTIBODIES AMONG PREGNANT WOMEN

Variable	n	IgM(+ve)	IgG(+ve)
	Age		
15-19	17	06	14
20-24*	36	16	30
25-29	15	04	14
30-34	16	07	14
35-39	06	01	03
≥40	00	00	00
	Trimester		
First	11	04	10
Second	23	10	20
Third	56	25	45
	History of Miscarriage		
Yes	27	13	21**
No	63	27	56
	History of MMR Vaccination		
Yes	0	0	0
No	90	34	75
	Experience of Rashes		
Recent	90	34	20
Past	90	56	75

*** x^2 Cal = 4.22, P=0.05; ** x^2 Cal 38.38, P =0.05**

TABLE 2: MARKED SURGE IN ANTI-RUBELLA IGG ANTIBODY AMONG PREGNANT WOMEN

Sample No	Rubella G Index of Convalescent(RGIC)	Rubella G Index of Pre-vaccination(RGIP)	Ratio of RGIC/RGIP
17	3.150	0.9	3.5
23	3.527	0.9	3.9
24	4.435	0.9	4.9
31	5.532	0.9	6.1
54	3.561	0.9	3.9
79	3.536	0.9	3.9
80	3.381	0.9	3.7
81	3.092	0.9	3.4
86	3.054	0.9	3.3
87	3.728	0.9	4.1
89	3.158	0.9	3.5
96	3.117	0.9	3.4
99	3.364	0.9	3.7
144	3.096	0.9	3.4

Manufacturer: RGIC/RGIP should be >1.5 to be suggestive of significant rise in antibody level.

DISCUSSION

This is the first serological evaluation of combined IgM and IgG antibodies among pregnant women in Maiduguri, Nigeria. The result of this study reveals 37.8% and 83.3% IgM and IgG seroprevalence respectively. The 37.8% IgM prevalence in this study is high compared to the 14.3%, 3.9%, 10%, and 16.3% prevalence in Abuja, Benue, Benin and Ilorin respectively (2, 8, 9) while the 83.3% IgG prevalence obtained in this study is also comparatively higher than the 53%, 62.7%, 68.5% prevalence in Benin, Zaria and Ibadan (9, 11, 12, 13,). Bukbuk *et al*., (2002) had earlier reported a 54.1% IgG seroprevalence in Maiduguri, but eleven years on, the prevalence of 83.3% in this study implies that more women have been and are perhaps still being exposed to rubella virus.

The age-stratified prevalence of anti-rubella antibodies among the pregnant women within the 20-24years revealed the highest prevalence of 40% (36/90)(x^2 Cal 4.22, p=0.05). We observed also that a combination of the 15-19years and 20-24years age bracket constituted a 58.88% of the pregnant women sampled in this study. This is instructive in that it shows that majority of the women in the area of study enter into childbearing early i.e. between 15-24years of age. This is in consonance with the tradition of early marriage in the northern part of Nigeria. None of the pregnant women sampled in this study was up to forty years of age. This is not unexpected since at such age, in northern Nigeria, many a woman would have become a grandmother due to early marriage.

The predominance of first time visitors (62.22%) to antenatal clinics at the third trimester of pregnancy was observed in this study (Table 1). This implies that majority of pregnant women either do not attend at all or present late at the antenatal clinics for routine medical attention. This attitude would impact negatively on any possible future effort to conduct surveillance on the prevalence of, and mitigate occurrence of children born with congenital rubella

syndrome in the study area in particular and Nigeria in general. This highlights the utmost importance of educating women of childbearing age in the study area on the need to enroll immediately in antenatal clinics whenever they are pregnant.

Rubella virus infection usually causes a mild disease in humans, but infection during early pregnancy often leads to severe congenital abnormalities (15). Therefore, the fetuses of 36.4% (4/11) of the pregnant women in the first trimester who were seropositive for IgM are at risk of severe congenital abnormalities. It also implies that had the pregnant women in the second trimester (76.9%; 10/13) and those in the third trimester (80.6%; 25/31) seropositive for IgM contracted this virus earlier, their fetuses would have also been at great risk of malformation (Table 1).

In this study, 10% (9/90) of the pregnant women were IgM and IgG anti-rubella antibodies negative. This means that they constitute population at risk. They are susceptible to infection by rubella virus. Among these nine, three (33.3%; 3/9) had history of pregnancy loss. This is indicative of miscarriage of non-rubella aetiology. The loss could only be attributed to other teratogenic organisms. However, 23.33% (21/90) of the pregnant women seropositive for rubella IgG had history of miscarriage while Chi-square analysis (x^2 Cal 38.38, p=0.05// x^2 tab 31.41, p=0.05) revealed an association between miscarriage and IgG antibody level in these category of pregnant women. This further corroborates the assertion by previous researchers that miscarriage is a sequelae of rubella virus infection.
Six point seven percent (6.7%; 6/90) of the pregnant women were IgM seropositive and IgG negative teratogenic agent responsible for several unreported cases of its sequelae- congenital rubella syndrome.

We recommend, therefore, that relevant authority devise drastic measure to be taken to protect women of childbearing age and fetuses from this scourge by, beside other measures, including rubella vaccination in routine antenatal screening exercise in Nigeria. There is also the need to health-educate women of childbearing age on how they could protect themselves from contracting this virus.

inferring that these pegnant women are undergoing acute infection. None of them was found to be in the first trimester of pregnancy, although two (2/6) of them had history of pregnancy loss. Even though they were IgM positive, rubella virus could not have been responsible for the past pregnancy loss due to the IgG seronegative status.

A marked surge in anti-rubella IgG antibody level was observed in fourteen of ninety pregnant women sampled (15.55%; x^2 Cal 19.85, p=0.05 // x^2 tab 22.36, p=0.05). This marked rise in antibody level is the ratio between the Rubella G Index of convalescent sample and that of pre-vaccination sample, which should be greater than 1.5 (Diagnostic Automation, Inc, USA). Manufacturer's manual specified Rubella G Index of 0.9 or less to be seronegative for IgG antibody to Rubella virus. None of the pregnant women in this study had any history of Rubella vaccination, therefore we assume pre-vaccination value to be 0.9 while the Rubella G Index of convalescent sample (after natural exposure, as the case in this study) is value obtained after analysis of sera (Table 3). The high ratio obtained for this samples (Table 3) is suggestive of recent infection even in the absence of IgM antibodies especially had further analysis revealed IgG with high avidity index. Due to scarce resources, however, avidity indices for these IgG antibodies were not determined.

In conclusion, the result of this work has revealed that rubella virus is still in circulation in the study area and that more women have been and are perhaps still being exposed to this potentially hazardous

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REFERENCES

1. Morbidity and Mortality Weekly Report: Three Cases of Congenital Rubella Syndrome in the Post elimination Era — Maryland, Alabama, and Illinois, 2012. March 29, 2013 / 62(12); 226-229

2. Enya, B., Effiong, M. A., Moffat, U. S. and Emem, B. (2011). Incidence of rubella IgM antibodies in individuals with febrile rash illness attending clinics in Akwa Ibom State, Nigeria, 2006-2009. *Health*, 3(6): 362-365

3. Junaid, S. A., Akpan, K. J. and Olabode, A. O. (2011). Sero-survey of rubella IgM antibodies among children in Jos, Nigeria. ***Virology Journal*** 8:244

4. Ching-Chiang, L., Chun-Yuh, Y., Ching-Tang, S., Bai-Hsiun, C., Yeou-Lih, H. (2010). **Rubella Seroepidemiology and Catch-up Immunization among Pregnant Women in Taiwan: Comparison between Women Born in Taiwan and Immigrants from Six Countries in Asia.** *Am J Trop Med Hyg*, **82**(1)**:**40-44.

5. Dontigny, L., Arsenault, M.Y., Martel, M.J., Biringer, A., Cormier, J., Delaney, M., Gleason, T., Leduc, D., Martel, M.J., Penava, D., Polsky, J., Roggensack, A., Rowntree, C. and Wilson, A.K. (2008). **Rubella in pregnancy.** *J Obstet Gynaecol Can*, 30152-158.

6. Shaheen, R., Kakru, D.K., Kauser, R., Gaash, B. and Hussain, S.N. (2008) Seroprevalence of rubella antibod-ies in Kashmiri pregnant women. *Indian J. of the Practising. Doc.*, **5**, 5-6.

7. Gomwalk, N.E. and Ahmed, A.A (1989). **Prevalence of rubella antibodies in the African continent.** *Rev. of infect. Dis.*, **11:**116-21.

8. Pennap, G., Amauche, G., Ajoge, H., Gabadi, S., Agwale, S. and Forbi, J. (2009). Serologic Survey of Specific Rubella Virus IgM in the Sera of Pregnant Women in Makurdi, Benue State, Nigeria. *Afr J Reprod Health*;13:69-73.

9. Onakewhor, J. U., and Chiwuzie, J. (2011). Seroprevalence survey of rubella infection in pregnancy at the University of Benin Teaching Hospital, Benin City, Nigeria. *Niger J Clin Pract*;14:140-5

10. Agbede, O.O., Adeyemi, O.O., Olatinwo, A.W.O., Salisu, T.J. and Kolawole, O.M. (2011). Sero-Prevalence of Antenatal Rubella in UITH. *The Open Public Health Journal* 4, 10-16

11. Oyinloye, S.O., Ahmad, A. A, Jatau, E.D. (2009). Seroprvalence of rubella virus infection in Zaria, Nigeria: An association between antibody level and community setting, *Biological and Environmental Sciences Journal for the Tropics* 9, 56-58

12. Bamgboye, A.E., Afolabi, K.A., Esumeh, F.I., Enweani. I.B.(2004). Prevalence of rubella antibody in pregnant women in Ibadan, Nigeria. *West Afr J Med*; 23: 245-8.

13. Bukbuk, D.N., EL-Nafaty, A.U., Obed, J.Y.(2002). Prevalence of rubella specific IgG antibody in non-immunized pregnant women in Maiduguri, North Eastern Nigeria. Cent. *Eur J Public Health* 10(1-2): 21-3.

14. Adeyemi, O.O., Agbede, O.O., Kolawole, M.O. and Okoh, A. (2012). Knowledge, attitude, and practices of pregnant women attending university of Ilorin Teaching Hospital with regard to rubella. Journal of Family and Reproductive Health (6)4: 153-158

15. Jila, S., Hasan, E. and Kazem, M. (2005) Congenital rubella syndrome in Iran. *BMC Infectious Diseases*, **5**, 44. doi:10.1186/1471-2334-5-44

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COMPARISON OF RAPID DIAGNOSTIC TESTS AND MICROSCOPY FOR MALARIA

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ABSTRACT

Presumptive treatment of malaria results in significant overuse of antimalarials. This study compared the diagnostic accuracy of Histidine Rich Protein II and plasmodium lactate dehydrogenase (pLDH)-based Rapid Kits(RDTs)and using expert microscopy as the gold standard for the detection of falciparum and non-falciparum in 200 individuals suffering from fever episodes over a period 8months in a malaria-endemic area in Osogbo, Osun State. 99(44.5%) of these patients were microscopically parasitaemic with three *Plasmodium species* identified expect *P.ovale*. 25 (12.5%) of the study population had temperature < 37.5^{0}C at the time of presentation in the clinic among which 16 (64%) were parasitaemic. Furthermore, 148 (74%) of the study population had fever episode of which 65(44%) were positive for malaria. The sensitivity and specificity of pLDH (*Pf*) were 84.7% and 78.3% respectively and HRP-2 were 72.7% and 90.9% respectively. Both had high detection (94.7%) at parasite density ≥ 10,000 parasite/µl of blood. Microscopy still remains the 'Gold Standard' since both are not 95% sensitive and cannot determine parasites quantification.

Keywords: Plasmodium, Microscopy, Rapid Kits, Osogbo, Nigeria, LAUTECH

COMPARAISON DES TESTS DE DIAGNOSTIC RAPIDE ET MICROSCOPIE POUR LE PALUDISME

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RÉSUMÉ

Le traitement présomptif de paludisme résulte de l'usage abusif considérable des antipaludiques. Cette étude a pour but de comparer l'efficacité de diagnostic de l'histidine RichProtein II et de test de diagnostic rapide (TDR) à base de kits plasmodium lactate déshydrogénase (pLDH) et en utilisant la microscopie experte comme «gold standard» pour la détection de *P. falciparum* et non-*falciparum* chez 200 personnes souffrant d'épisodes de fièvre sur une période de huit mois dans une région où le paludisme est endémique dans Osogbo, l'Etat d'Osun. 99 (44,5%) de ces patients étaient parasitémiques à la microscopie à trois espèces de *Plasmodium*identifiées différentes de *P. ovale*attendu. 25 (12,5%) de la population étudiée avait une température <37,5°C au moment de leur arrivée à la clinique parmi lesquels, 16 (64%) étaient parasitémiques. En outre, 148 (74%) de la population d'étude avait un épisode de fièvre dont 65 (44%) étaient positifs pour le paludisme. La sensibilité et la spécificité de pLDH (Pf) étaient respectivement de 84,7% et 78,3% et celles de HRP-2 étaient respectivement de 72,7% et 90,9%. Tous les deux

tests avaient une bonne détection (94,7%) à densité parasitaire ≥ 10000 parasite/ul de sang. La microscopie reste le «Gold Standard» puisque les deux autres tests ne sont pas sensibles à 95% et ne peut pas déterminer la quantité parasitaire.

Mots clés: Plasmodium, microscopie, kits de test rapide, Osogbo, Nigeria, LAUTECH

INTRODUCTION

Malaria remains an important public health concern in countries where transmission occur regularly as well as in areas where transmission has been largely controlled or eliminated. An estimated 40% of the World population today is at risk of malaria infection and the World Health Organization (WHO) estimates that each year there are more than 300 millions episodes of acute illness and at least two million deaths due to Malaria (1). Malaria is a complex disease that varies widely in epidemiology and clinical manifestations in different parts of the world. Variable factors such as distribution and efficiency of mosquito vector, climate and other environmental conditions and the behavior and level of acquired immunity of the exposed human population contributes to wide distribution of malaria(1).Methods for diagnosis of malaria in endemic countries include microscopy, RDT, polymerase chain Reaction (PCR), and clinical methods. Microscopy remains the gold standard diagnostic technique of choice for malaria. It is less costly and sensitive to a threshold of 5 to 50 parasite /µl (depending on the microscopist expertise). It can also characterize the infecting species and their relative densities (2). Above all, microscopy requires considerable technical expertise for optimal blood film preparation, examination and interpretation. Immunochromatographic capture procedure is an RDT based on the detection of malaria antigen and was developed to improve the timeless, sensitivity and objectivity of malaria diagnosis through less reliance on expert microscopy, (2). Preferred targeted antigens are those which are abundant in all asexual and sexual stage of the parasite.

Currently interest is focused on the detection of histidine-rich protein2 (HRP-2) from *Plasmodium falciparum* and parasite-specific lactate dehydrogenase (pLDH) or Plasmodium aldolase from the parasite glycolytic pathway found in all species. However, several factors in the manufacturing process as well as environmental conditions may affect RDT performance. These include sub-optimal sensitivity at low parasite densities, an inability to accurately identifying parasites to the species level or quantify infection density, and a higher unit cost relative to microscopy (2).

Presumptive / clinical diagnosis is the least expensive and most commonly used method and is the basis for self-tropical diseases like typhoid fever, respiratory tract infections and viral infections impairs its specificity and therefore encourages the indiscriminate use of antimalarials for managing febrile conditions in endemic areas. Accuracy of a clinical diagnosis varies with the level of endemicity, malaria season and age group. No single clinical algorithm is a universal predictor (3).Changing patterns of accepted morphological appearance of malaria species, possibly due to drug pressure, strain variation, or approaches to blood collection, have created diagnostic problems that cannot easily be resolved merely by reference to an Atlas of Parasitology (4).

WHO currently recommends that parasite based diagnosis should be used in all cases of suspected malaria with the possible exception of children in high prevalence areas and return traveler from endemic zones (Samuel *et al.,* 2008 5).Prompt and accurate diagnosis is a key to effective treatment and management of patients with malaria parastemia which will eventually reduce malaria morbidity and mortality. This work is designed to compare the current methodologies and approaches in the diagnosis of malaria in a practical and helpful way for the laboratory and for the physician caring for the patients.

MATERIALS AND METHODS

Study area: This study was conducted in LAUTECH teaching hospital, Osogbo.Osogbo metropolis is in Osun state and is located in South-west of Nigeria.Malaria is endemic in these areas and predominant during the raining season. Mean annual rainfall 1250-2000mm with relative humidity of 60-70% and temperature of 28-32°C and a population density of 448,000 ***(2009 census)***.

Subject selection: The symptomatic individual of different ages were recruited in this study. Febrile patients with typical malaria symptoms (headache, joint pains, body weakness) both inpatients and outpatient of these hospitals were recruited into the study. Inclusion criteria include fever (temperature ≥37.5°C,) and other malaria symptoms like headache, joint pains, body weakness and diarrhoea.

Ethical issues and clearance: Ethical approval was obtained from ethical committee of LAUTECH Teaching hospital, Osogbo.

Patients and Sample collection: The period of sample collection was 7months January-August,2013 (ending of dry season and beginning of raining season) The biodata of 200 subjects were noted and included age,

sex. Also clinical data such as history of fever in the past 24 hours, headache, generalized body pain, joint pains, chills and rigor were noted. Consenting febrile patients with auxiliary temperature of >37.5^{0}C were recruited into the study and five millimeters of blood was collected from the antecubital vein area of the patients after cleaning the area with methylated spirit. Part of the blood was used for microscopic examination of malaria parasite.

Laboratory procedure

Blood film Microscopy: Thick and Thin blood films were made following standard laboratory procedure and the malarial parasite were counted in relation to number of white blood cells (WBC) usually 200WBC (or 500WBC) when the number of parasites is less than 10 per 200 WBC counted and multiplying this by the average of the total WBC counted in such individual as earlier described by Onile and Taiwo , 2005.(6)

Rapid Diagnostic Tests

The SD BIOLINE kit (**SD** Bioline System Korea) which contains a plastic cassettes which is pre-coated with two monoclonal antibodies as two separate lines across the test cassettes. One monoclonal antibody (test line 2) is pan-specific to lactate dehydrogenase (pLDH) of the Plasmodium species(*P.falciparium, P.vivax, P.malariae, P.ovale*) and the other line (test line 1) consists of a monoclonal antibody specific to histidine-rich protein 2 (HRP2) of the *P. falciparum* species and *one step malaria Histidine-rich protein II(*P.f)test were used for the study. This was carried out and interpreted following manufacturer's instructions.Internal procedural controls were included in the test.

Method for presumptive Diagnosis

Method for clinical diagnosis was based solely on clinician's judgment who based the diagnosis on presenting complaints and from physical examination of the patient without any reference to laboratory test/analysis. Some of the symptoms presented are, fever which is the chief complain, vomiting, headache, anorexia, abdominal pain, body pain, diarrhea, Tiredness, sweating, diarrhea

The temperature of each patient was noted together with other symptoms presented. Majority of patients with temperature ≥37.5°C were recruited in to the study.

Analysis of Results

Data were analyzed using SPSS packaged version 16.0. Sensitivity refers to the proportion of the samples with positive result. Specificity refers to the proportion of the samples with negative result.The present data were calculated using the formulas:

Sensitivity = TP/(TP + FN) 100%

Specificity = TN/(TN + FP) 100% : where TN represents true negative, TP true positive, FN false negative and FP false positive .

RESULTS

A total of 200 patients with clinical symptoms of malaria were enrolled in this study. It was observed in the age and gender distribution among the study population;that the female gender was more represented in all the age groups, with the 1-20 years old group having the highest frequency as shown in figure 1. These differences were not significant (X^2 = 3.47, df =3, p = 0.433) as shown in Table 1. Figure 2 compares the age group with parasitaemia, 137 (68.5%) within the age group 1-20 had the highest parasitaemia 82 (59.9%). However the differences were significant (X^2= 18.93, df =3, p= 0.001). Table 2 shows the clinical presentation among the patients in which fever is prevalent. 65 (44%) of the total number 148 that complained of having fever were actually observed to be parasitaemic.

A body temperature of ≥37.5^{0}C was recorded in 175 cases (87.5 %) of which 83 (45%) were positive for malaria while the rest were negative. Those withtemperature ≤37.5^{0}C were 25 (12.5%9) among which 16 (64%) were positive for malaria. The difference in the number of patients having either of the two temperature group significantly affect those that were parasitaemic (X^2 = 7.39, p= 0.002).

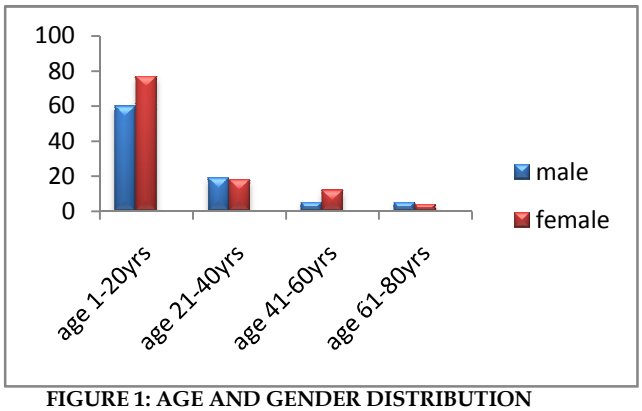


FIGURE 1: AGE AND GENDER DISTRIBUTION AMONG THE STUDY POPULATION

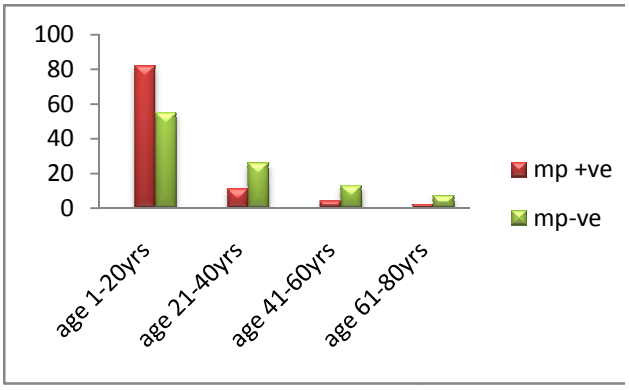


FIGURE 2: RELATIONSHIP BETWEEN AGE GROUP AND PARASITAEMIA

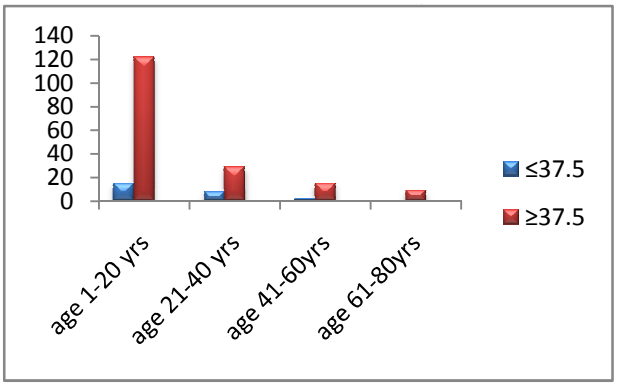
The relationship between clinical body temperature among the study gender within age groups as shown in figure 3.The difference between age and temperature was not statistically significant (X^2= 4.41, df= 3 p= 0.220) even though there was no significant difference in temperature presentation and sex group (X^2= 0.142, df= 1, p= 0.707).

The species of *Plasmodium falciparum* was detected in 84 (40%) of the total blood samples collected with density ranging between 43 and 227,857 parasites/µl of blood (mean= 3712.07).From figure 4 9 (9.09%) of the positive samples had a mixed infection of *P.vivax*and *P.falciparum* having a density of 94-680/µl (mean= 5.91/µl). Onlysix samples had a *P.malariae*and *P.falciparum* withdensity ranging between 57and 331parasites/µl of blood (mean= 2.77/µl).

Comparison Of Microscopy With Rdts

The result of the performance of the RDTs with the reference standard of microscopy is indicated in table 3, 4 and 5. The prevalence of Plasmodium species using microscopy and RDTs was 81 (40.5 %), 85 (42.5 %), 41 (%) and 99 (44.5 %) respectively as indicated in Table 3.A composite reference was generated and used as the gold standard to assess the sensitivity of each method used in the analysis. This was defined as true positive if all the two methods tested positive and as true negative, if all the two methods tested negative. Sensitivity is defined as the probability that a truly infected individual will test positive, and specificity as the probability that a truly uninfected individual will test negative (Table4).

TABLE 3 : RELATIONSHIP AGE GROUP & TEMPERATURE



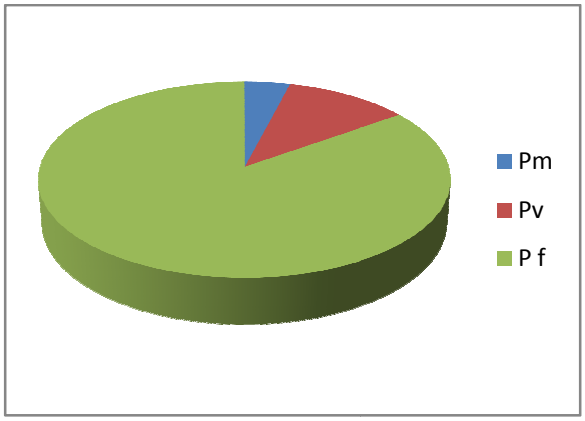


FIGURE 4: *PLASMODIUM SPECIES* POPULATIONS

The pLDH (*pf*) was more sensitive than SD Bioline (84.7%) although the latter was more specific (90.9%). SD Bioline showed more false negative result (29) than pLDH ( ) (Table 5).

pLDH was commercially made to detect other plasmodium species causing malaria infection. Even though made specifically to detect HRP-2 antigen expressed by *P. falciparum and Pan* ,having high percentage detection (90%) and 0% at parasite density of < 100 and where as SD Bioline has percentage detection of 55 %. Both assays are very sensitive at higher parasitaemia.

TABLE 1: AGE GROUP AND SEX DISTRIBUTION OF PATIENTS AMONG THE STUDY POPULATION

Age group (years)	Gender Female	Male	Total	P value
1-20	**77**	**60**	**137**	**0.433**
21-40	**18**	**19**	**37**	
41-60	**12**	**5**	**17**	
61-80	**4**	**5**	**9**	
Total	**111**	**89**	**200**	

TABLE 2: PREVALENCE AND CLINICAL PRESENTATION OF MALARIA AMONG THE STUDY POPULATION

Clinical Symptoms	Number Observed	No. of microscopy positve (%)	No. of RDTS positive	
			HRP 2 (%)	**pLDH (%)**
Fever	148	65 (44)	54 (37)	56 (38)
Headache	121	38 (32)	31 (27)	35 (29)
Chills	113	47 (42)	39 (35)	43 (38)
Cough	36	5 (19)	4 (11)	7 (19)
Tiredness	27	9 (33)	4 (15)	6 (22)
Diarrhoea	18	12 (67)	5 (28)	3 (17)
Anorexia	32	16(50)	11(34)	8 (25)
Vomiting	31	12 (39)	7 (23)	14 (45)
Abdominal pain	10	2 (22)	3 (30)	4 (40)
Body pain	43	15 (35)	6 (14)	5 (12)
Myalgia	12	7 (58)	5 (42)	8 (67)
Sweating	41	13(32)	7 (17)	10 (24)

TABLE 3: PREVALENCE OF MALARIA ACCORDING TO DIFFERENT DIAGNOSTIC METHODS USED.

	No Positve	% Positive	No Negative	% Negative
Microscopy	99	44.5	101	55.5
HRP II	81	40.5	119	59.5
pLDH (pan)	41	20.5	159	79.5
(*Pf*)	85	42.5	115	57.5

TABLE 4: COMPARISON OF DIAGNOSTIC TEST RESULTS OF THE HRP2, PLDH AND MICROSCOPY

Result/Methods	Microscopy	HRP II	pLDH	Composite Reference
True positive	72	72	72	72
False positive	27	9	13	_
True Negative	90	90	90	90
False Negative	10	29	25	_

TABLE 5: SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE AND NEGATIVE PREDICTIVE VALUE OF THE THREE TESTS.

	Microscopy	HRP 2	pLDH
Sensitivity(%)	87.8	72.7	84.7
Specificity(%)	76.9	90.9	78.3
Positive Predictive value(PPV%)	72.7	88.9	84.7
Positive Predictive value(NPV%)	90	75.6	78.3

DISCUSSION

The people suffering from malaria among the study population were almost average although they had clinical manifestation, which may presumably be symptoms of malaria infection (Table 1). From this study, the female gender were more than the male (Figure 1) because birth statistic shows that female children are more than male. Among other age groups female presented themselves for malaria for malaria diagnosis more than male counterpart due to cultural believe and drug abuse (concoctions). In addition female had temperature ≥ 37.5^{0}C within the gender age group of 1-20 and 41-60. This could be as a result of physiological changes especially during their menstruation periods and menopause (Hot flushes). The high prevalence of parasitaemia observed among the children of age group 1-20 is suggestive of underdeveloped immunological status.

Fever, headache, chill and sweating in order of prevalence which was clearly observed among the study population confirmed the febrile paroxysm of malaria presentation which is likely to coincide with schizogonic cycle of *Plasmodium* in the human host (7).Although many had temperature greater than 37.5^{0}C at the time of presentation in the clinic for diagnosis, this may be due immunological response to presence of antigens such as *Plasmodium* and other disease causative agents (co-infection). But the few that had temperature < 37.5^{0}C could be as a result of typical periodicity of paroxysm which lasts for 72 hours in *P.malariae* and 48 hours in other species, although sometimes continuous in *Falciparum* malaria (7).

The presence of Plasmodium species in the body system elicits host immune responses in which TNF-α, IL- 1 and other cytokines were secreted which in the process of mounting up resistance, up-regulates endothelia cell and cause fever (8). Hence, the higher prevalence in fever for both parasitaemia and antigenaemia. However, fever symptom cannot categorically be allied as a measure for clinical diagnosis of malaria as it serves as a generalized symptom for most diseases.

The accuracy and urgency in the diagnosis of malaria made the use of rapid diagnostic method imperative especially in rural areas where health facilities and electric power supply are inadequate, two rapid diagnostic kits of the same product were (SD Bioline pLDH for the diagnosis of *Plasmodium falciparum* andother speciesand HRP-2) were evaluated for their efficacy.

From the study SD Bioline pLDH (*Pf / Pan*) and HRP-2 were found to be very easy to use and the result can be visibly read in 15 minutes. The limitation of this kit is that there is a greater risk of infection, if there should be spill from the mixture in the well unlike other diagnostic kit in which the blood and diluents are applied into the cellulose pad directly. However, the ability of both kits to detect parasite antigens (Histidine Rich Protein -2 and Plasmodium Lactase dehydrogenase respectively) from finger prick blood allow efficient handling for use by non-technician with less training in agreement with findings from other studies (9,10).

The prevalence (20.5%) of pLDH (*Pan)* test for non-falciparum species was low compare with *Pf* (42.5%) and HRP -2 (44.5%) this is because other species of plasmodium are not common in this area however *P. falciparum* demands particular attention because it is the species of human malaria parasite that carries the most severe form of the disease and can kill with stunning speed.

Gametocytes were detected by microscopy in only 3 of the 200 cases (1.5%) no HRP 2 and pLDH results were positive in a case with the smear that showed gametocytes. *P.vivax* and *P.malariae* was also detected by microscopy which were picked by the PLDH. From this finding, SD Bioline was found to be more sensitive (Table 5) and pLDH made specifically to detect HRP-2 antigen expressed by *P. falciparum has* high percentage detection at parasite density of < 100 than HRP-2. However both performed excellently at higher parasite density > 10,000/µl .

The specificity of both assays are encouraging despite the occurrence of occasional false positive *P. falciparum* detection. This could probably be due to previous recent infection with malaria or the presence of circulating rheumatoid factor and some report has also establish the decreased activity assays with antimalaria therapy (11).

REFERENCES

1. Center for Disease control and Prevention. About Malaria. 2010. Your online source of credible health information.
2. Geofrey E Playford and John Walker (2002): Evaluation of ICT malaria P.F/P.V and the optimal Rapid Diagnostic Tests for malaria in febrile Returned Travellers. Journal of clinical microbial. Nov. 2002 p.4166-4171.
3. Wongsrichanalai, C.,Mazic J.Barcus Sinuon Muth, Awalludin Sutamihardja and Walther H, Werisdore 2007. A review of Malaria Diagnostic Tools. Microscopy and Rapid Diagnostic Test (RDT) America Journal of Tropical Medicine Hyg **77:**119.
4. Beadle, C., G. W. Long, W. R. Weiss, P. D. Meelroy, S. M. Maret, A. J. Oloo,and S. L. Hoffman. 2004. Diagnosis of malaria by

detection of *Plasmodium falciparum* HRP-2 antigen with a rapid dipstick-capture assay. Lancet, **i:**564–568.

5. Samuel Shilcutt,Chantal Morel,Catherine Goodman, Paul Coleman, David Bell, ChristopherJ.M,Whitty and A Mills.2008 Cost-effectiveness of malaria diagnostic methods in Sub-Saharan Africa in an era of combination theraphy.Bulletin of the world health organization 86:101.
6. Onile,B.A, Taiwo,S.S 2005: Recent Advances in the Laboratory Diagnosis of Malaria. Africa Journal of Clinical and Experimental Microbiology AJCEM/2004040/2519./ 6 (2): 113 - 123
7. Reyburn H, Mbatia R, Drakeley C, Carneiro I, Mwa Kasungula E, Mwerinde O: Overdiagnosis of malaria among patients with severe febrile illness in Tanzania : a prospective study. BMJ, 2004**;329**:1212 do:10,1136/bmj.38251658229.55.
8. Chen Q, Schlichtherle M, Wahlgren M. (2000)**.** Molecular Aspect of Severe Malaria. Clinical Microbiology Review. Page 439-450.
9. Agomo P.U., Okonkwo C.A., Asisanya., Ania O.O., Okoh H.I and Nebe O.J.(200). Comparative Evaluation of Immunochromatograhic Test (ICT) and parasight for Rapid Diagnosis of *falciparum* Malaria in Nigeria. America Journal of Clinical Experimental Microbiology. 2(1):43-51
10. Playford E. Geoffrey and Walter John (2002).Evaluation of the ICT Malaria *Pf / P.v* and the optimal rapid Diagnostic Tests for Malaria in Febrile Returned Travelers. *Journal of Clinical Microbiology,* 40(11):4166-4171.
11. Iqbal, J., A. Sher, and A. Rab**.** 2000. *Plasmodium falciparum* histidine richprotein-2 based immunocapture diagnostic assay for malaria: cross reactivity with rheumatoid factors. J. Clin. Microbiol. **38:**1184–1186.

ORIGINAL ARTICLE

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THE PREVALENCE OF INTESTINAL COCCIDIAN PARASITES BURDEN IN HIV/AIDS PATIENTS ON ANTIRETROVIRAL THERAPY IN HIV CENTERS IN MUBI, NIGERIA

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ABSTRACT

Background: Intestinal coccidia are group of protozoa which parasitize the epithelial cells of the intestinal tract of their hosts. Most infections usually produce mild, self-limiting infections in man, but they now constitute a serious public health problem, especially in developing countries with inadequate sanitary conditions coupled with widespread HIV/AIDS infection.
Objective: To determine the Prevalence of intestinal coccidian parasites burden in HIV/AIDS patients on antiretroviral therapy in HIV Centers in Mubi, Nigeria
Materials and Methods: This was a hospital-based cross-sectional study in which stool specimens from HIV-positive patients on ART were examined for the presence ofoocysts of intestinal coccidian parasitesusing Modified Acid Fast Stain technique. In addition, patients' blood samples were analyzed for CD4 count by flow cytometry and packed cell volume (PCV) through microhaematocrit centrifugation.

Results A total of 305 specimens examined, 236(77.4%) were positive for *Cryptosporidium parvum*, *Isospora belli* and *Microsporidium* species. Patients within the age group of 21 – 30 were the most infected. Generally, the duration of ART influenced the prevalence of the intestinal coccidian parasites. There was a highly significant association between the CD4 count and prevalence coccidian parasites ($p < 0.05$). There was a significant negative correlation ($r = -0.95$) between the duration of the ART and the prevalence of coccidian presence.

Conclusion: Routine screening of HIV-positive patients for intestinal parasites is advocated as standard operative procedure (SOP) before antiretroviral therapy (ART) is given. Construction of public health facilities, toilets and boreholes as well as public enlightenment campaign is recommended for more effective management of these patients.

Keywords: intestinal coccidian parasites, antiretroviral therapy, Mubi

LA PREVALENCE DES COCCIDIES INTESTINALES CHEZ LES PATIENTS ATTEINTS DE VIH/SIDA SOUS TRAITEMENT ANTIRETROVIRAL DANS LES CENTRES DE CONTROLE DE VIH DE MUBI AU NIGERIA.

Djieyep[1], A C N., Djieyep[2], F D., Pokam[3], B T., David[4], D L., and Kamga[5*] H L F.

Résumé
Contexte: Les coccidies intestinales sont un groupe de protozoaires qui parasitent les cellules épithéliales du tube digestif de leurs hôtes. La plupart des infections humaines sont d'habitude peu sévères et autolimitées, mais elles constituent de nos jours un véritable problème de santé publique, particulièrement dans des pays en voie de développement où les conditions sanitaires sont inadéquates, et couplées à l'infection répanduedu VIH/SIDA.

Objectif: Déterminer la fréquence de coccidies intestinales chezles patients atteints de VIH/SIDA sous traitement antirétroviral dans les Centres de contrôle de VIH de Mubi au Nigeria.
Matérielset Méthodes: Il s'agissait d'une étude transversale dans laquelle les spécimens de selles des patients séropositifs au VIH et sous traitement antirétroviralétaient examinés en vue d'en dépister là la présence d'oocystes de coccidies intestinales grâce à la technique de de coloration acido-résistante modifiée. De plus, les prélèvements de sang des patients étaient analysés pour en déterminer le taux de CD4 et le taux demicro-hématocrite par les techniques de flux cytométrique et de centrifugation respectivement.
Résultats: Untotal de 305 spécimens ont été examinés, 236 (77.4 %) étaient positifs pour le *Cryptosporidiumparvum*,le *Isospora belli* et les espèces de Microsporidie. Les patients dans la tranche d'âge de 21 – 30 ans étaient les plus infectés. Généralement, la durée du traitement antirétroviralinfluençait la fréquence des coccidies intestinales. Il y avait une association fortement significative entre le taux de CD4 et les infections($p< 0.05$). Il existait une corrélation négative significative ($r =-0.95$) entre la durée detraitement antirétroviralet la fréquence de coccidies intestinales.
Conclusion: Le dépistage de routine des patients séropositifs pour des parasites intestinaux est préconisé dans la procédure opératoire standard avant toute administration de thérapie antirétrovirale. La construction d'installations de santé publique, des toilettes et des puits de forage ainsi que des campagnes d'éducationsanitaire sontfortement recommandées en vue d'une prise en charge effectivedes patients atteints de VIH.
Mots-clés : coccidies intestinales, thérapie antirétrovirale, Mubi

INTRODUCTION

Gastrointestinal tract (GIT) infections constitute a serious public health problem in developing countries with inadequate sanitary conditions coupled with widespread HIV/AIDS infection. Many different types of intestinal parasites infect man, provoking a wide range of symptoms that are generally associated with gastrointestinal tract disorders and are dependent on demographic, socio-economic, physiological and immunological factors [1]. With the progressive development of AIDS, especially once $CD4^{+}$Tlymphocyte counts have fallen below 200 cells/µl, patients often become co-infected by bacteria, parasites or viruses [2]. Gastrointestinal tract parasitic infections often present as diarrhea and significant disease has been recorded in 50-96% of cases with 90% prevalence rate reported in Africa [3].Infective causes of chronic diarrhea may satisfactorily be managed because with the exception of cryptosporidiosis and HIV-related enteropathy, good response to treatment can be expected [4], but all etiologic agents are not easily diagnosed in Africa on routine basis because of limited diagnostic facilities and trained personnel [5].

Following HIV infection, the virus replicates rapidly in lytic cycles inside resident tissue macrophages and $CD4^{+}$ T lymphocytes. This results in depletion of these vital immune cells, rendering the individual susceptible to opportunistic pathogens, a host of which include intestinal protozoa, resulting in the frequently observed diarrhea in this group of persons [6].The incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community [7].Several parasites have been implicated as major contributors to morbidity in HIV-infected persons living in developing countries, and the parasites frequently encountered include mostly intestinal coccidia such as: *Cryptosporidium* spp., *Isospora belli* and*Microsporidia* spp [8]. Most of these infections in man are zoonotic (having the potential to infect animals or arise from animals). In immunocompetent individuals, they usually produce mild, self-limiting infections [9].

Cryptosporidium parvum is the species responsible for human infections and is now widely recognized as a cause of acute gastro-enteritis, particularly in children and other immunosuppressed persons such as AIDS patients [10]. The infection produces a persistent, watery, offensive diarrheic stool, often accompanied by abdominal pain, nausea, vomiting and anorexia. In immunocompetent persons, symptoms are usually short lived (1 to 2 weeks). The small intestine is the site most commonly affected. In immunocompromised individuals, especially in HIV patients, Cryptosporidiosis can be life threatening. As many as 10% of AIDS patients pass oocysts of *C. parvum* in their stools[11].*Isospora belli* has a cosmopolitan distribution; occurring especially in warm regions of the world infecting both humans and animals [12].It is endemic in Africa, Asia and South America. Infection with *I. belli*manifest as darrhea with abdominal cramp which can last for weeks and result in fat malabsorption and weight loss. In immunocompromised individuals, infants and children, infection ranges from self-limiting enteritis to severe diarrhea illness resembling that of cryptosporidiosis [13]. *Cyclospora cayetanensis*has also been described in association with diarrhea illness but the infection usually results in a disease with non-specific symptoms [14].*Microsporidia species* are known as opportunistic pathogens in humans with Acquired Immune Deficiency Syndrome (AIDS) andhave

beenimplicatedinconditions ranging from enteritis to keratoconjunctivitis [15].The most common *Microsporidia* found in patients with AIDS are *Enterocytozoon bieneusi, Encephalitozoon intestinalis* and *Encephalitozoon hellem*.Patients with these infections tend out to be severely immuno-deficient with a $CD4^+$ T cell count less than 100 x 10^6/L [16]. In addition, cases of microsporidiosis have been reported in immunocompromised persons not infected with HIV and in immunocompetent individuals. The clinical manifestations of microsporidiosis are very diverse, varying according to the causal species with diarrhea being the most common [16].

Since the prevalence of parasites associated with gastroenteritis is dependent of their geographical distribution and socio-economic factors surrounding a given community, laboratory investigations are required to determine prevalence in each population in order to provide an effective planning and management policy.Human immunodeficiency virus (HIV) and parasites have long been of public health concern and have drawn global attention. However, although much work have been done on the prevalence of parasites in HIV patients, at the moment there is scarcity of information on the prevalence of intestinal coccidia among HIV positive patients in Mubi HIV centers of Adamawa State, Nigeria. The availability of such data will be an enormous contribution to the implementation of regulatory policies on the management of HIV/AIDS patients, hence this study was conducted.

MATERIALS AND METHODS

Study area

Adamawa state is located in the North Eastern part of Nigeria. It lies between latitude 7˚ and 11˚ north of the equator and longitude 11˚ and 14˚ east of the Greenwich Meridian. It is divided into 21 Local government areas [17].The growth of Mubi town is traced to the agricultural, administrative, and commercial functions it performs. The hospitals in which this study was carried out arethe three HIV specialized centers of the Mubi General Hospital which geo-politically covers Mubi North, Mubi South, and Michika Local Governments.The Hospitals care for the people living with HIV and also receives patients from other parts of the country due to their consistent supply of reagents for CD4+ counts for HIV infected patients.

Sample collection

The study was cross-sectional hospital based study carried out from June to October 2013. Male and female HIV/AIDS patients visiting the hospital for treatment or routine follow-up were provided with information on the purpose/objectives of the study andits possible benefits. They were however explained that it was not an obligation for them to participate in the research and neither was it a pre-requisite to accessing routine medical or other social services publicly available. The effective study participants (ESP) were those whoconsented and provided stool samples for laboratory investigation and their blood samples were taken for $CD4^+$ T cell determination.

Freshly voided stool samples were collected into clean wide mouth specimen containers from volunteer participants. A portion of the stool was preserved in 10% formalin in a proportion of 10g of stool in 3 mL of formalin. Five mL of venous blood were collected into coated EDTA tubes for $CD4^+$ T cell counts and packed cell volume (PCV).The ESP were grouped based on their levels of immune suppression thus: Based on the $CD4^+$ T cell counts, the participants were categorized by their immune status according to the 1993 Revised Classification System for the HIV Infection by CD4 T-cell categories [18].Samples were numbered progressively for identification and these laboratory numbers ensured individual confidentiality.Participants were not billed for the tests and the physicians were provided with the findings for necessary action. The samples collected for the study as resolved with the participants were solely used for the defined purpose. The ethical clearance was approved by the Ethical Committee of the Hospital prior to sample collection.

Modified Ziehl Neelson Stain

Detection of intestinal coccidian parasitesoocysts in the concentrated stool was done using the modified cold Ziehl Neelsen staining technique. A concentrated smear of the stool was made on a clean grease-free slide and fixed in methanol for 3 minutes. The slide was immersed in cold Carbol fuchsin and stained for 15 minutes. It was then thoroughly rinsed in tap water and decolorized in 1% HCl (v/v) in methanol for 10-15

minutes. After rinsing again in tap water, the slide was counterstained with 0.4% malachite green for 30 seconds. The slide was then air-dried and observed under the compound light microscope using 40x objective lens for the presence of *Cryptosporidium* oocysts, which was confirmed under the oil-immersion objectives as small pink to red spherules on pale green background.

Determination of CD4⁺ T cell count

Blood samples collected from the same patients who provided stool samples were analyzed for CD4⁺ T lymphocyte cell estimation using flow cytometry. Briefly, 20 µl of CD4 PE antibody was placed into a Partec test tube and 20µl of well-mixed whole EDTA blood was added, mixed gently and incubated in the dark for 15 minutes at room temperature. The mixture was agitated during incubation every 5 minutes. 800 µl of CD4 buffer was added to the mixture of antibody and sample and mixed gently. This was then plugged to the counter for counting.

Determination of the Packed Cell Volume (PCV)

The PCV was estimated by centrifuging a sample of well-mixed anticoagulated blood in capillary tubes using the microhaematocrit centrifuge. The machine was set at 12,000g for 5 minutes which automatically attains the correct speed. The PCV was subsequently estimated by measuring the height of the red cell column and expressing it as a ratio of the height of the total blood column. A PCV reader determined this ratio [19].

Data analysis

Data got from the processing of sampleswere analyzed using SPSS 12.0.The Chi-Square test was used to compare proportions at significant level of 0.05.

RESULTS

A total of 305 specimens examined, 236(77.4%) were positive for *Cryptosporidium parvum*, *Isospora belli* and *Microsporidium* species. Table 1 shows the prevalence of coccidian parasites in study participants according to gender. There was no significant difference in the prevalence by gender of participants ($P > 0.05$).

TABLE 1: PREVALENCE OF COCCIDIAN PARASITES IN STUDY PARTICIPANTS ACCORDING TO GENDER

Parasites	Number (%)of participant infected		TOTAL n=305
	Males (n=68)	Females(n =237)	
Cryptosporidium parvum	32 (47.0)	95 (40.1)	127(41.6)
Isospora belli	23 (33.8)	72 (30.4)	95(31.1)
Microsporidium species	2 (2.9)	10 (4.2)	12(3.9)
Total	57 (83.8)	177 (74.6)	234(76.7)

The Prevalence of coccidian parasites in study participants according to age is shown in Table 2. Patients within the age group of 21 – 50 were the most frequently infected, though there was no significant difference in the prevalence byage ($P > 0.05$).

TABLE 2: PREVALENCE OF COCCIDIAN PARASITES IN STUDY PARTICIPANTS ACCORDING TO AGE

Parasites	Number (%)of infected participants aged (years)			Total n=305
	<21 (n=30)	21—50 (n=239)	>51 (n=36)	
Cryptosporidium parvum	10 (33.3)	102(42.7)	15(41.7)	127(41.6)
Isospora belli	9 (30.0)	74 (30.9)	12(33.3)	95(31.1)
Microsporidium species	1 (3.3)	11 (4.6)	0	12(3.9)
Total	20 (66.6)	187 (78.2)	27(75.0)	234(76.7)

Table 3 shows the prevalence of intestinal coccidian parasite infection according to the duration of ART by patients. There was a highly significant association between the prevalence of intestinal coccidian parasites and to the duration of treatment by patients (P<0.05)

TABLE 3: PREVALENCE OF INTESTINAL COCCIDIAN PARASITE INFECTION ACCORDING TO THE DURATION OF ART

Parasites	Number (%) of patients having been treated for							Totaln = 305
	1 yr n = 85	2 yrs n = 80	3 yrs n = 60	4 yrs n= 30	5 yrs n = 30	6 yrs n = 5	7 yrs n = 15	
Cryptosporidium parvum	40(47.1)	19(23.8)	31(51.7)	17(56.7)	14(46.7)	1(20.0)	5(33.3)	127(41.6)
Isospora belli	27(31.8)	21(26.3)	21(35.0)	8(26.7)	10(33.3)	3(60.0)	5(33.3)	95(31.1)
*Microsporidium*species	7(8.2)	1(1.3)	2(3.3)	0(0)	1(3.3)	0(0)	1(6.7)	12(3.9)
Total	74(87.0)	41(51.1)	54(90.0)	25(83.3)	25(83.3)	4(80.0)	11(73.3)	234(76.7)

Table 4 shows the prevalenceof parasites in study participants according to CD4 count. There was a strong negative correlation (r = -0.95) between the duration of the ART and the prevalence of coccidian presence and the association between CD4 count of patients and the presence of coccidian parasites showed a high level of significance (P < 0.05)

DISCUSSION

In this study, there was a high prevalence (76.7%) of coccidian parasite infection in the study population conducted during . The fact that the study was conducted during the wet period could account for this unexpected high prevalence; Previous studies in Rwanda [20] and Kenya [21]have shown that peaks of infection tend to occur in the wet months. Reports from West Africa [22,23] and Zambia [24] have shown human infections peak early in the season, perhaps because susceptible populations develop immunity after repeated exposure with the initial rains. This high

prevalence can further be explained by the widespread of common open toilets, the incidence of random defecation, and the occurrence of stormy winds. It has been shown by Siobhan et al [25] that the storm water runoff during rainy seasons undoubtedly increases environmental transport of feces and wet, humid conditions favor parasite survival. Also the intensive rearing of cattle in the region and the use of ovine manure as fertilizer surely goes a long way to enhance the epidemiology of parasites. Moreover, the lack of hygiene can equally enhance the transmission of these parasites.Risk factors for acquisition of parasitic infections are the same in both immunocompetent (IC) and immunosuppressed (IS) individuals. The immune system plays an integral part in modifying the establishment of infection, controlling disease once it is established, limiting the severity and dissemination of the disease, and assisting in clearance or control of the parasite. Thus, immunosuppressed hosts are more likely to acquire infection after exposure, have more severe disease once the infection is established, have disseminated infection rather than localized infection, and be unable to clear parasites with chronic carriage states. These all lead to, and account for, the greater morbidity and mortality in these patients.

In our study, there was no significant difference in the prevalence by gender of participants. This finding disagrees with the report from Kenya [26]. The authors argued that that exposure to *Cryptosporidium* was influenced by gender, age and role in the household. Adult women had more daily contact with cattle faeces than adult men, and older women had more contact than older men. Women took more care of sick people and were more at risk from exposure by this route. We foundno significant difference in the prevalence of infection with coccidian intestinal parasite by age. This finding is not in accordance with a report from India [27] in which the highest prevalence of these organisms was in the group aged 16-45 years and during the rainy months. A similar study carried out in Israel [28] reported an Age-related infection with *Cryptosporidium* species. Since these infections may be asymptomatic or present as a mild self-limited disease, many infected peoples do not seek medical aid. Consequently, under-appreciation and over-appreciation of infection with intestinal coccidians may occur depending on the diagnostic technique used. Our study population comprised HIV-positive patients already on ART: it was not the case for the earlier-mentioned reports. That is probably the reason for the above disagreements.

TABLE 4: PREVALENCEOF PARASITES IN STUDY PARTICIPANTS ACCORDING TO CD4 COUNT

Parasites	Number (%) of infected patients with CD4 count			Totaln= 305
	<200 n=30	200 – 499 n=201	≥ 500 n= 74	
Cryptosporidium parvum	14(46.7)	92(45.8)	21(28.4)	127(41.6)
Isospora belli	11(36.7)	65(32.3)	19(25.7)	95(31.1)
Microsporidium species	0(0)	8(4.0)	4(5.4)	12(3.9)
Total	25(83.3)	165(82.1)	44(59.4)	234(76.7)

Since the patients involved in this study were all on ART, one could have expected a lower parasites load, since the treatment is supposed to have boosted their immune status. Thus, comparison in this study between the parasite load and the immune status of patients based on their $CD4^+$ T cell counts according to the 1993 Revised Classification System for the HIV infection by $CD4^+$ T-cell categories by Castro et al.[18] showed that 83.3% of the patients infected with the opportunistic coccidians were significantly associated with CD4 count below 200. This is in concordance with the view that the outcome of infection by enteric

protozoan parasites is dependent on absolute $CD4^+$T cell counts, with lower counts being associated with more severe disease [29].

The present study showed no significant difference in the intestinal profiles of study participants using wet preparation based on their $CD4^+$T cell counts. On the other hand, unlike the non-opportunistic parasites, low CD4 count (83.3% with <200 CD4 count) was highly significantly associated with the presence of coccidians, where there is a decrease in CD4 counts at the beginning of treatment and a higher prevalence of the parasites.

The mean PCV of the patients with CD4 count less than 200 was significantly lower compared to those with CD4 count within 200 and 500, and those with CD4 above 500. Though one cannot ascribe the patients in this study to be anemic, previous studies have shown that $CD4^+$T cell count of less than 200cells/µL were associated with an increased risk of parasitic infection among HIV-infected patients, and co infection with Malaria, a risk to anemia [30]. This is important as CD4 count of less than 200cells/ µL is believed to be associated with disease progression and opportunistic infections. This may explain the association between CD4 count and parasitic infections among HIV patients with anemia.

CONCLUSION

The prevalence of Coccidian parasites in HIV/AIDS patients attending Mubi HIV centres is relatively high.Female patients were more infected with commonly identified parasites through wet preparation while there was no significant difference in infection rates with opportunistic parasites. The age range most affected in this study was between 21 to 40 years, with the peak between 21 to 30 years for the opportunistic coccidians.As the duration of treatment increased, the parasitic load decreased.There was no significant difference in the intestinal profiles of study participants using wet preparation while the prevalence of patients infected with the opportunistic coccidians was highly significantly associated with CD4 count.There was no significant difference between CD4 count and PCV, though lower PCV was equally associated with low CD4 counts. Public health measures should continue to emphasize the importance of environmental and personal hygiene as well as provide and monitor the source and quality of drinking water. This is a very important point as the community depends a lot on wells as sources of water and the prevalent wind storms disseminate the pathogens throughout the localities. Stool processing should be a routine in HIV/AIDS patients attending treatment centers and more training should be given to health practitioners and laboratory technicians about opportunistic emerging parasites.

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AUTHORS' CONTRIBUTIONS

Armand Claude Noundo Djieyepconceived and designed the study, conducted the literature search, drafted the manuscript and carried out the laboratory investigations. Felicité Djieyep Djemna assisted in the design and the laboratory investigations. Delphine Leila Davidsupervised the research work. Benjamin Thumamo Pokamparticipated in the literature search.Henri Lucien Kamga substantially revised the manuscript and prepared it for publication. All authors read and approved the manuscript.

COMPETING INTERESTS

The authors declare having no competing interests.

REFERENCES

[1]. Mohammed, A., Gabre-Selassie, S., Tesfaye, K. and Gabre, K. Prevalence of intestinal parasites in HIV infected adult patients in South Western Ethiopia. Ethiop Journ Dev 2003,17(1): 71-78.

[2]. Zheng, X.Y. Acquired immunodeficiency syndrome prevention and control in China. Clin JournEpidemiol1999, 20: 131–134.

[3]. Gurerrant, R. and Boback, D. Bacterial and Protozoal Gastroenteritis. New EnglJourn Med 1990, 325 (5): 327-340.

[4]. Framm, S. and Soave, R. Agents of diarrhoea. Med ClinJourn North Am 1997,81: 427-447.

[5]. Orterga, Y., Sterling, C., Gilman, R., Cama, V. and Diaz, F. Cyclospora species-new protozoan pathogens of humans. New EnglJourn Med1993, 328: 1308-1312.

[6]. Kaleebu, P., French, N. and Mahe, C. "Effect of human immunodeficiency virus (HIV) type 1 envelope subtypes A and D on disease progression in a large cohort of HIV-1-positive persons in Uganda". Journ Infect Dis2002, 185 (9): 1244–1250.

[7]. Mannheimer, S.B. and Soave, R. Protozoal infections in patients with AIDS. Cryptosporidiasis, Cyclosporiasis and Microsporidiasis.Infec Dis Clin Journ North Am 1994,8:483-498.

[8]. Lucas, S.B. Missing infections in AIDS. Trans Royal Soc Trop MedHyg1990, 84: 34–38.

[9]. Lewethwaite, P., Gill, G., Hart, C. and Beeching, N. Gastrointestinal parasites in the immunocompromised. Curr Op Infec Dis 2005, 18: 427-435.

[10]. Bruce, F. and Mark, F. Gastrointestinal diseases: Pathophysiology, Diagnosis and Management of GIT Disorders. 1993, 5th ed. USA: WB Saunders, 54:225-260.

[11]. **O'Donoghue, P.** *Cryptosporidium* **and Cryptosporidiosis in Man and Animals. Intern Journ Parasitol 1995, 25(2):111-122.**

[12]. Sorvillo, F., Lieb, L., Seidel, J., Kerndt, P., Turner, J. and Ash, L. (1995) Epidemiology of Isosporiasis among persons with Acquired Immunodeficiency Syndrome in Los Angeles. AmJourn Trop Med Hyg 1995,53:656–659.

[13]. Garcia, L.S.H. Diagnostic Medical Parasitology 2004,3rd Edition, California, ASM press.

[14]. Herwaldt, B. *Cyclospora cayetanensis:* Review, focusing on the outbreaks of cyclosporiasis in the 1990s. Clin Infec Dis 2000, 31:1040-1057.

[15]. Didier, E. and Weiss, L. Microsporidiosis:current status. Curr Op InfecDis, 2006, 19: 485-492.

[16]. Davis, A., Haque, R. and Petri, W. (2002). Update on protozoan parasites of the intestine. Curr Op Gastroenterol, 18:10-14.

[17]. Adebayo, A. A. and Tukur, A. L. Climate II, Rainfall. Adamawa State in Maps 1999. Paraclete Publishers, Yola. Pp 22-23.

[18]. Castro, K., Ward, J., Slutsker,L., Buehler, J., Jaffe, H. and Ruth, L. (1993). Revised classification system for HIV infection and expanded surveillance on definition of AIDS among adolescents and adults. Morb Mort Weekly Rep 1993, 41: 1-19.

[19]. Baker, F. J. and Silverton, R.E. Measurement of PCV by centrifugation. Introduction to Medical Laboratory Technology. 1985, Sixth Edition. Butterworth & Co (Publishers) Ltd. Pp327-328.

[20]. Bogaerts, J., Lepage, P., Rouvroy, D. Cryptosporidiosis in Rwanda: clinical and epidemiological features. Ann Soc Belge Med Trop 1987, 67:157–165.

[21]. Gatei, W., Wamae, C.N. and Mbae, C. Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. Am Journ Trop MedHyg,75:78–82.

[22]. Molbak, K., Hojlyng, N. and Gottschau, A. Cryptosporidiosis in infancy and childhood mortality in Guinea Bissau, West Africa. Bissau MedJourn 1993, 307:417–420.

[23]. Adegbola, R. A., Demba, E., De Veer, G. and Todd, J. *Cryptosporidium* infection in Gambian children less than 5 years of age. Journ Trop MedHyg 1994, 97:103–107.

[24]. Nchito, M., Kelly, P. and Sianongo, S. Cryptosporidiosis in urban Zambian children: an analysis of risk factors. Am Journ TropMed Hyg 1998, 59:435–437.

[25]. Siobhan, M. and Tziposi, S. Cryptosporidiosis in Children in Sub-Saharan Africa: A Lingering Challenge. Clin Infec Dis 2008, 47(7): 915–921

[26]. Kimani VN, Mitoko G, McDermott B, Grace D, Ambia J, Kiragu MW, Njehu AN, Sinja J, Monda JG, Kang'ethe EK. Social and gender determinants of risk of cryptosporidiosis, an emerging zoonosis, in Dagoretti, Nairobi, Kenya. Trop Anim Health Prod. 2012 Sep;44 Suppl 1:S17-23.

[27]. Gopal Nath A, Choudhury B.N, Shukla T.B, Singh and D.C.S. Reddy. Significance of *Cryptosporidium*in Acute Diarrhoea in North-Eastern India J. Med. Microbiol. 1999, 48 : 523-526

[28]. Miron D, Colodner R and Kenes Y. Age-related seroprevalence of cryptosporidium in Northern Israel. IMAJ 2000, 2:343-345

[29]. Stark, D., Barratt, J. L. N., Van Hal, S., Marriott, D., Harkness, J. and. Ellis, J. T. Clinical Significance of Enteric Protozoa in the Immunosuppressed Human Population. Clin Micro Rev 2009, 22 (4): 634-650.

[30]. Akinbo, F.O., Okaka, C.E., Omoregie, R. and Igbinumwen, O. Prevalence of malaria and anaemia among HIV-infected patient in Nigeria. New Zeal Journ of MedLabSc 2009, 63: 78-80.