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Prof Boaz Adegboro (MD)
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3. Arya, O. P., Osoba, A. O., and Bennett, P. Tropical Venereology, Churchill Livingstone, Edinburgh, 1980 OR when referring to a chapter in a book and where the names of authors are also given, the reference should be as follows:


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EBOLA VIRUS DISEASES (EVD)

*Elisha, A. & Adegboro, B.

Department of Medical Microbiology, LAUTECH Teaching Hospital, Ogbomoso, Oyo State, Nigeria.

*Correspondence: Rusha91@msn.com +234-815-993-8343

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. However, the current outbreak, which started 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. 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)

*Elisha, A. & Adegboro, B.

Département de Microbiologie Médicale, Centre Hospitalier Universitaire LAUTECH, Ogbomoso, Etat d’Oyo, Nigéria.

*Auteur correspondant: Rusha91@msn.com +234-815-993-8343

RESUME


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 as 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**

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

VIROLOGY

Ebola Virus is closely related to the Marburg virus, and they both belong to Filoviridae family. The family name came from their characteristic 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 nucleocapsid 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. 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 membrane(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).

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 organisms such as EBV, but their role in EBVD is not well defined. Wilson et al (2000) vaccinated mice with Venezuelan equine encephalomyelitis 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-I 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, lymphadenopathy, 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 orbitis, 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 immunosorbent 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 seroconversion 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 *Hypsiprymnathus monstosus*, *Eptomops franqueti*, and *Myonycteris 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**

POLYMERASE CHAIN REACTION (PCR) PROVIDES A SUPERIOR TOOL FOR THE DIAGNOSIS OF PNEUMOCOCCAL INFECTION IN 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.1; Traoré/Ouedraogo, R.3,4; Hatcher, C.5; Mayer, L.5; Wang, X.5; Sangaré, L.1,3.

1Service de Bactériologie-Virologie CHU YalgadoOuedraogo, 03 BP 7022 Ouagadougou 03, Burkina-Faso; 2Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles, Université de Ouagadougou, Burkina Faso, 03BP 7131 Ouagadougou 03; 3UFR Sciences de la Santé (UFR-SDS), Université de Ouagadougou, 03 BP 7021 Ouagadougou 03, Burkina Faso; 4Laboratoire de Bactériologie-virologie, CHU Charles de Gaulle, Ouagadougou, Burkina Faso; 5Division of Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA

* Correspondence: Chaibou, Y., Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles, Université de Ouagadougou, Burkina Faso, 03BP 7131 Ouagadougou 03, Fax: +22650337373, Tél:+22678048600. Email: cyaou@hotmail.com

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.

Material 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 (51.2%) were positive for pneumococci by latex agglutination assay and culture. These specimens were tested with rt-PCR, which confirmed 36.4% (140/385) Gram positive encapsulated diplococci; 37.7% (145/385) and 20.8% (80/385) of the specimens were positive by rt-PCR. 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.1; Traoré/Ouedraogo, R.3,4; Hatcher, C.5; Mayer, L.5; Wang, X.5; Sangaré, L.1,3.

1Service de Bactériologie-Virologie CHU YalgadoOuedraogo, 03 BP 7022 Ouagadougou 03, Burkina-Faso; 2Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles, Université de Ouagadougou, Burkina Faso, 03BP 7131 Ouagadougou 03; 3UFR Sciences de la Santé (UFR-SDS), Université de Ouagadougou, 03 BP 7021 Ouagadougou 03, Burkina Faso; 4Laboratoire de Bactériologie-virologie, CHU Charles de Gaulle, Ouagadougou, Burkina Faso; 5Division des infections bactériennes, Centres de Contrôle et de Prévention des Maladies, Atlanta, Georgia, Etats-Unis.

* Adresse Mail d’auteur correspondant: Chaibou, Y., Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles, Université de Ouagadougou, Burkina Faso, 03BP 7131 Ouagadougou 03, Fax: +22650337373, Tél:+22678048600. cyaou@hotmail.com

RESUME

Objectif: Le but de cette étude était de déterminer la place de la rt-PCR dans la surveillance de routine de méningites pneumococciques au Burkina Faso et la comparée avec les méthodes de la bactériologie classique: Culture, coloration de Gram et l’agglutination au latex.

Matériel et méthodes: Au total, 385 échantillons de liquides 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.
INTRODUCTION

*Streptococcus pneumoniae* (pneumococcus) is a common pathogen associated with benign or severe infections including acute otitis media, meningitis, sepsis, and pneumonia (1). According to WHO annual reports, 1.6 million cases of fatal pneumococcal disease occurred worldwide 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. pneumoniae* is fastidious and sensitive to temperature variation and therefore requires rapid and accurate identification for proper and timely antibiotic therapy and epidemiologic surveillance. Bacterial etiology is typically identified and characterized using standard methods such as Gram stain, latex agglutination assay, culture, susceptibility to optochin (ethyl hydrocurreine 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 method was 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). To overcome the limitations of the standard bacteriological methods, molecular methods were 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*, *Haemophilus influenzae* serotype b and *S. pneumoniae* simultaneously in an effort to evaluate the usefulness of PCR technology in meningitis surveillance (12). In another study, PCR identified 27% pneumococci from CSF specimens but culture and latex agglutination assay only 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 Department of the Teaching Hospital Yalgado Ouedraogo (TH-YO) in Ouagadougou, Burkina Faso. The specimens were collected from nine medical regions of Burkina Faso: Center, Centre-West, Center-South, Center-North, Central Plateau, East, North, Boucle of Mouhoun and the Sahel, from March 2010 to December 2012.

**Clinical specimen collection**

The cerebrospinal fluid (CSF) was obtained from patients with suspected meningitis. A case of suspected 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 fontanel and 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 suspected meningitis and three or five milliliters (3-5 mL) of CSF specimen were collected and transferred into an sterile tube for laboratory confirmation. The specimens collected at the teaching hospital of Ouagadougou were transported to the laboratory in less than one hour after collection. For the specimens coming from other medical regions, 1 mL of CSF was inoculated into Trans-Isolate (T-I) medium for culture and 0.5 mL 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 were observed.

Latex agglutination assay

The PASTOREXTM Meningitis 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, 1 or 2 drops of CSF specimen inoculated T-I medium was plated onto chocolate agar plate and/or onto 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 and/or 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 equal or larger than 14 mm were 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, produced 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. pneumoniae 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) and 75U/ml of mutanolysin (Sigma-M9901) was pipetted into themicrocentrifuge tube. Then, 200uL 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-rtPCR, 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 using 12.5µl 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 >35 cycles and ≤40 cycles, and the negative result as a Ct value was ≥40 cycles. All inconclusive or equivocal results were diluted to 1:4 and 1:10 and 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 385 CSFs specimens were collected from suspet cases for meningitis. S. pneumoniae was detected in 204 (51.2%) CSFs specimens, by the three methods (latex agglutination assay, culture and rt-
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. pneumoniae* 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

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive (%)</th>
<th>95%CI</th>
<th>Negative (%)</th>
<th>95%CI</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>140 (36.4)</td>
<td>31.2-40.9</td>
<td>245 (63.6)</td>
<td>56.2-74.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>145 (37.7)</td>
<td>32.4-42.2</td>
<td>240 (62.3)</td>
<td>55.7-74.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Culture</td>
<td>80 (20.8)</td>
<td>16.6-24.8</td>
<td>305 (79.2)</td>
<td>69.3-92.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>rt-PCR</td>
<td>197 (51.2)</td>
<td>44.8-54.9</td>
<td>188 (48.8)</td>
<td>45.1-55.2</td>
<td>0.6835</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Combination of methods</th>
<th>Number detected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rt-PCR+/Culture+/Latex agglutination assay+</td>
<td>76 (19.7)</td>
</tr>
<tr>
<td>rt-PCR+/Culture-/Latex agglutination assay+</td>
<td>62 (16.1)</td>
</tr>
<tr>
<td>rt-PCR+/Culture-/Latex agglutination assay-</td>
<td>57 (14.8)</td>
</tr>
<tr>
<td>rt-PCR-/Culture+/Latex agglutination assay+</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>rt-PCR-/Culture+/Latex agglutination assay-</td>
<td>0</td>
</tr>
<tr>
<td>rt-PCR-/Culture-/Latex agglutination assay+</td>
<td>5 (1.3)</td>
</tr>
<tr>
<td>rt-PCR+/Culture+/ Latex agglutination assay-</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>204 (53.0)</td>
</tr>
</tbody>
</table>

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 7 CSFs 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 the negative 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

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

### 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 standard bacteriological methods (Gram stain, culture and latex agglutination assay) 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 PCR is 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, only 20.4% 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 sensitivity was 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 this situation, 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 *Streptococccus* species and not specific to *S. pneumoniae* especially in carriage studies (25). PCR assays targeting genes such as * sodA* (superoxide dismutase A gene) (26), * sp9802* (fragment 9802 gene) (21), and *psaA* (pneumococcal surface adhesion A gene) (25, 27) were less specific than pneumococcal *lyt* PCR (15, 17, 28, 29). Our data showed that rt-PCR targeting autolysin gene *lytA* was a complementary method for identification of *S. pneumonia* during the epidemic seasons in Burkina Faso.
### TABLE 4: SENSITIVITY AND SPECIFICITY OF DIFFERENT METHODS

<table>
<thead>
<tr>
<th>Method</th>
<th>Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>132</td>
<td>26</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>64</td>
<td>163</td>
<td>227</td>
</tr>
<tr>
<td>Gram stain</td>
<td>Total</td>
<td>196</td>
<td>189</td>
<td>385</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>140</td>
<td>9</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>59</td>
<td>177</td>
<td>236</td>
</tr>
<tr>
<td>Latex agglutination assay</td>
<td>Total</td>
<td>204</td>
<td>186</td>
<td>390</td>
</tr>
<tr>
<td>rt-PCR</td>
<td>Positive</td>
<td>197</td>
<td>7</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>181</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>197</td>
<td>188</td>
<td>385</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>67.4</td>
<td>86.2</td>
<td>83.5</td>
<td>71.8</td>
</tr>
<tr>
<td>Latex agglutination assay</td>
<td>70.4</td>
<td>95.2</td>
<td>70.4</td>
<td>75.0</td>
</tr>
<tr>
<td>Culture</td>
<td>54.4</td>
<td>31.5</td>
<td>32.9</td>
<td>52.8</td>
</tr>
<tr>
<td>rt-PCR</td>
<td>96.6</td>
<td>100</td>
<td>100</td>
<td>96.3</td>
</tr>
</tbody>
</table>

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 these methods 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 bacterium and to determine 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 agglutination assay 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 specificity of latex agglutination assay of 96% and 85% respectively (17). These results prove the importance of latex agglutination assay in 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 standard bacteriological methods, particularly the culture that contribute to 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 Faso allows confirmation of more suspect cases of pneumococcal meningitis compared to culture and latex 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.

**ACKNOWLEDGEMENTS**

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

Iyoha, O. and Tula, M.Y.

Department of Medical Microbiology, School of Medicine, College of Medical Science, University of Benin, P.M.B. 1152, Benin City, Nigeria; Department of Biological Science Technology, Federal Polytechnic Mubi, P.M.B. 35, Adamawa State, Nigeria.

*Correspondence: E-mail: drosaiyoha@yahoo.co.uk. Tel: +2348023352188

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%), cloxacin (67%), ofloxacin (54%), ciprofloxacine (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

Iyoha, O. et Tula, M.Y.

*Département de Microbiologie Médicale, Ecole de Médecine, Faculté de Science Médicale, Université de Bénin, P.M.B. 1152, ville de Bénin, Nigéria; Département de Science de TechnologieBiologique, Polytechnique Fédérale de Mubi, P.M.B. 35, Etat de Adamawa, Nigéria.

*Correspondance: E-mail: drosaiyoha@yahoo.co.uk. Tél: +2348023352188

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 uréral (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 Klebsiella oxytoca,Citrobacter spp et Streptococcus spp étaient les moindres (0.67%). Ils ont été testés pour la sensibilité à 9 antibiotiques. Le taux de résistance observé était le...
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, 

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°C for 24hrs. Commercially available antimicrobial discs used included: ciprofloxacin (30µg), ceftriaxone (1µg), ofloxacin (25µg), augmentin (25µg), cefuroxime (30µg), gentamycin (10µg), cefazidime (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.

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%).


**TABLE II: ANTIBIOGRAM OF ISOLATES (%)**

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

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 (%)**

<table>
<thead>
<tr>
<th>No. of organisms</th>
<th>Percentage of organisms</th>
<th>No. of antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>4.7</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>14.0</td>
<td>4</td>
</tr>
<tr>
<td>29</td>
<td>19.3</td>
<td>5</td>
</tr>
<tr>
<td>40</td>
<td>26.7</td>
<td>6</td>
</tr>
<tr>
<td>39</td>
<td>26.0</td>
<td>7</td>
</tr>
<tr>
<td>14</td>
<td>9.3</td>
<td>8</td>
</tr>
<tr>
<td>150</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

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...
This result is contrary to the findings 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 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 cephaporin antibiotics as well as β-lactam/β-lactamase inhibitors. This study in contrast observed low resistance rate towards ceftriaxone (38%) and cefix (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, cefotaxime, ceftriaxone and cefoxitin (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. aureas exhibits remarkable versatility in their behaviour towards antibiotics [41]. Outbreaks of S. aureas 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 bacteria is naturally resistant 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, sepsis, 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 cefotaxime. The strains were also highly resistant 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

REFERENCES


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

Bissong, M. E. A., Fon, P. N., Kamga, F. H. L. & Akenji, T. N.

1Department of Microbiology and Parasitology, Faculty of Science, University of Buea, Cameroon; 2Department of Public Health, Faculty of Health Sciences, University of Buea, Cameroon; 3Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Bamenda, Cameroon

AUTEUR POUR CORRESPONDANCE: Marie Ebob Agbortabot Bissong, Département de Microbiologie et Parasitologie, Faculté des Sciences, Université de Buea. BP 63, Buea, Cameroon. Tel: +237 75301641 Email: mabissong@yahoo.com

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 diabetics. 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 BUCALES CHEZ LES PATIENTS DIABÉTIQUES ET TEMOINS NON DIABÉTIQUES DU SUD-OUEST, CAMEROUN

Bissong, M. E. A., Fon, P. N., Kamga, F. H. L. & Akenji, T. N.

DÉPARTEMENT DE MICROBIOLOGIE ET PARASITOLOGIE, FACULTÉ DES SCIENCES, UNIVERSITÉ DE BUEA, CAMEROUN; DÉPARTEMENT DE SANTÉ PUBLIQUE, FACULTÉ DES SCIENCES DE LA SANTÉ, UNIVERSITÉ DE BUEA, CAMEROUN; DÉPARTEMENT DES SCIENCES DU LABORATOIRE MÉDICAL, FACULTÉ DES SCIENCES DE LA SANTÉ, UNIVERSITÉ DE BAMENDA, CAMEROUN

AUTEUR POUR CORRESPONDANCE: Marie Ebob Agbortabot Bissong, Département de Microbiologie et Parasitologie, Faculté des Sciences, Université de Buea. BP 63, Buea, Cameroon. Tel: +237 75301641 Email: mabissong@yahoo.com

RÉSUMÉ
Contexte: La flore microbienne orale est de plus en plus incriminée dans les infections bucales. 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 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 que 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 oral; infections buccales; diabète; Cameroun

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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 linked 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°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 \textit{Candida albicans} from other \textit{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 \( P \) \textless 0.05 were considered statistically significant.

\section*{RESULTS}

\subsection*{A. Distribution of Oral Isolates among Study Participants}

\begin{table}[h!]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Mouth Isolates & Diabetics n (%) & Non-diabetics n (%) & Total n (%)
\hline
\textit{Candida albicans} & 30 (19.5) & 15 (13.6) & 45 (17.0)
\textit{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)
\textit{Acinetobacter Spp} & 0 (0.0) & 1 (0.9) & 1 (0.4)
\textit{Burkholderia} & 2 (1.3) & 0 (0.0) & 2 (0.8)
Other \textit{Candida} Spp & 12 (7.8) & 6 (5.5) & 18 (6.8)
\textit{E. coli} & 1 (0.6) & 0 (0.0) & 1 (0.4)
\textit{Enterobacter Spp} & 2 (0.1) & 0 (0.0) & 2 (0.8)
\textit{Flavimonas Spp} & 0 (0.0) & 1 (0.9) & 1 (0.4)
\textit{GNR*} & 8 (5.2) & 16 (14.5) & 24 (9.1)
\textit{Klebsiella Spp} & 8 (5.2) & 7 (6.4) & 15 (5.7)
CNS & 14 (9.1) & 3 (2.7) & 17 (6.4)
\textit{Providencia Spp} & 0 (0.0) & 1 (0.9) & 1 (0.4)
\textit{Serratia Spp} & 10 (6.5) & 9 (8.2) & 19 (7.2)
\textit{Kluyvera Spp} & 1 (0.6) & 0 (0.0) & 1 (0.4)
Total ** & 154 (58.3) & 110 (41.7) & 264 (100)
\hline
\end{tabular}
\caption{Distribution of oral isolates in diabetics and non-diabetics}
\end{table}

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

\subsection*{B. Comparison of Oral Isolates between Diabetics and Non-Diabetics}

\begin{figure*}[h!]
\centering
\caption{Comparison of oral isolates between diabetics and non-diabetics}
\end{figure*}

\textbf{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 (\textit{Candida} spp) from the oral cavity between diabetics and non-diabetics (\( \chi^2 \)-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 (\( \chi^2 \)-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

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

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

<table>
<thead>
<tr>
<th>Oral Isolates</th>
<th>Oral disease</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gingivitis n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Periodontitis n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dental caries n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida</em> spp</td>
<td>11 (28.9)</td>
<td>P=0.75</td>
<td>14 (35.9)</td>
<td>P=0.393</td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td>38 (100)</td>
<td>-</td>
<td>39 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td>12 (31.6)</td>
<td>P= 0.17</td>
<td>12 (30.8)</td>
<td>P = 0.61</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>39</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

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
Other studies have demonstrated that diabetes mellitus might enhance oral candidal colonization (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.

ACKNOWLEDGEMENT

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Conflict of interest Statement: The authors declared no conflict of interest in the present manuscript.

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

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

Babajide TI, Nwadike VU, Ojo DA, Onasanya OA, Ojide KC, Kalu IE

1. Dept of Microbiology, Federal University of Agriculture Abeokuta. 2. Division of Medical Microbiology

Dept of Pathology Medical Center Abeokuta. 3. Dept of Public and allied health Babcock University Ilishan Ogun state. 4. Dept of Medical Microbiology FTH Abakaliki. 5. Dept of Medical Microbiology FMC Umuahia.

Correspondence: email: victornwadike@yahoo.com

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.

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).

M. 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 Umua hia 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 \textit{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: \textit{MYCOBACTERIUM TUBERCULOSIS} INFECTION IN RELATION TO AGE

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Total number examined (%)</th>
<th>\textit{Mycobacterium tuberculosis} infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (%)</td>
</tr>
<tr>
<td>0-20</td>
<td>18 (13.6)</td>
<td>4 (3.0)</td>
</tr>
<tr>
<td>21-40</td>
<td>64 (48.5)</td>
<td>15 (11.4)</td>
</tr>
<tr>
<td>41-60</td>
<td>40 (30.3)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>61-81</td>
<td>10 (7.6)</td>
<td>1 (8.0)</td>
</tr>
<tr>
<td>Total</td>
<td>132 (100.0)</td>
<td>22 (16.7)</td>
</tr>
</tbody>
</table>

$X^2= 6.752$ \hspace{1cm} $P= 0.080$

Table 3, Showed that the overall prevalence of \textit{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 \textit{M. tuberculosis} infection ($P= 0.880$)

Table 5 Showed the results of the cross-tabulation between \textit{M.tuberculosis} infection and Erythrocyte Sedimentation Rate (ESR). It showed that none of the patient with \textit{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**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total number examined (%)</th>
<th>Mycobacterium tuberculosis infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (%)</td>
</tr>
<tr>
<td>Male</td>
<td>72 (54.5)</td>
<td>14 (10.6)</td>
</tr>
<tr>
<td>Female</td>
<td>60 (45.5)</td>
<td>8 (6.1)</td>
</tr>
<tr>
<td>Total</td>
<td>132 (100.0)</td>
<td>22 (16.7)</td>
</tr>
</tbody>
</table>

\[X^2 = 0.880 \quad P = 0.348\]

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

<table>
<thead>
<tr>
<th>PCV (Range)</th>
<th>Total number examined (%)</th>
<th>Mycobacterium tuberculosis infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (%)</td>
</tr>
<tr>
<td>Normal (35-54)</td>
<td>94 (71.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Moderate (26-34)</td>
<td>21 (15.9)</td>
<td>5 (3.8)</td>
</tr>
<tr>
<td>Severe (&lt; 26)</td>
<td>17 (12.9)</td>
<td>17 (12.9)</td>
</tr>
<tr>
<td>Total</td>
<td>132 (100.0)</td>
<td>22 (16.7)</td>
</tr>
</tbody>
</table>

\[X^2 = 95.895 \quad P = 0.001\]

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

<table>
<thead>
<tr>
<th>ESR</th>
<th>Total number examined (%)</th>
<th>Mycobacterium tuberculosis infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (%)</td>
</tr>
<tr>
<td>2-10</td>
<td>107 (81.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>11-40</td>
<td>5 (3.8)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>41-70</td>
<td>9 (6.8)</td>
<td>9 (6.8)</td>
</tr>
<tr>
<td>71-100</td>
<td>11 (8.3)</td>
<td>11 (8.3)</td>
</tr>
<tr>
<td>Total</td>
<td>132 (100)</td>
<td>22 (16.7)</td>
</tr>
</tbody>
</table>

\[X^2 = 112.218 \quad P = 0.001\]

**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, a lower prevalence of M. tuberculosis was recorded 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.* (19). 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**


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

*Oyinloye, S.O.1, Amama, C.A.1, Daniel, R.1, Ajayi, B. B.2, and Lawan, M.A.2

1Department of Microbiology, Faculty of Science, University of Maiduguri, Borno, Nigeria; 2Department of Immunology, University of Maiduguri Teaching Hospital, Borno, Nigeria.

*Correspondence: faisam26@gmail.com; 0803 616 1371

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 ($\chi^2$ Cal 5.1; p=0.05) and IgG respectively. Chi-square analysis ($\chi^2$ Cal 38.38, p=0.05/$\chi^2$ tab 31.41, p=0.05) revealed an association between miscarriage and IgG antibody level in twenty-one subjects. Pregnant women within 20-24 years had the highest prevalence of 40% (36/90) ($\chi^2$ Cal 4.22, p=0.05): 44.4% (16/36) of them were seropositive for IgM ($\chi^2$ Cal 4.31, p=0.05). A marked surge in IgG antibody level, which tantamount acute infection, was observed in 15.6% (14/90) ($\chi^2$ 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

*Oyinloye, S.O.1, Amama, C.A.1, Daniel, R.1, Ajayi, B. B.2, and Lawan, M.A.2

1Département de Microbiologie, Faculté de Science, Université de Maiduguri, Borno, Nigéria; 2Département d’Immunologie, Université de Maiduguri, Centre Hospitalier Universitaire, Borno, Nigéria.

*Correspondence: faisam26@gmail.com; 0803 616 1371

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 ($\chi^2$ Cal 5.1, p=0.05) et IgG de la rubéole. L’analyse Chi-carré ($\chi^2$ Cal 38.38, p=0.05/$\chi^2$ Tab. 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) ($\chi^2$ Cal 4.22, p=0.05): 44.4% (16/36) d’entre elles étaient séropositives pour les IgM ($\chi^2$ 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) (X² 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 limited 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-epidemi 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°C in the Department of Immunology, University of Maiduguri Teaching Hospital until analysis was done.

**Specimen Analysis**

The Enzyme linked immunosorbert 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 15-19 years and 20-24 years 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 40 years 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>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>IgM(+ve)</th>
<th>IgG(+ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-19</td>
<td>17</td>
<td>06</td>
<td>14</td>
</tr>
<tr>
<td>20-24*</td>
<td>36</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>25-29</td>
<td>15</td>
<td>04</td>
<td>14</td>
</tr>
<tr>
<td>30-34</td>
<td>16</td>
<td>07</td>
<td>14</td>
</tr>
<tr>
<td>35-39</td>
<td>06</td>
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<td>03</td>
</tr>
<tr>
<td>≥40</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td><strong>Trimester</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>11</td>
<td>04</td>
<td>10</td>
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<tr>
<td>Second</td>
<td>23</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Third</td>
<td>56</td>
<td>25</td>
<td>45</td>
</tr>
<tr>
<td><strong>History of Miscarriage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27</td>
<td>13</td>
<td>21**</td>
</tr>
<tr>
<td>No</td>
<td>63</td>
<td>27</td>
<td>56</td>
</tr>
<tr>
<td><strong>History of MMR</strong></td>
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</tr>
<tr>
<td>Vaccination</td>
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<tr>
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<td>34</td>
<td>75</td>
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<td>Recent</td>
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</tr>
<tr>
<td>Past</td>
<td>90</td>
<td>56</td>
<td>75</td>
</tr>
</tbody>
</table>

* $x^2$ 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

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

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 20-24 years revealed the highest prevalence of 40% (36/90) (x^2 Cal 4.22, p=0.05). We observed also that a combination of the 15-19 years and 20-24 years 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 15-24 years 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
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 inferring that these pregnant 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 childbearing age on how they could protect themselves from contracting this virus.

Acknowledgement
We are grateful to all the member of staff of Fatima Ali Sheriff Hospital, Maiduguri for helping in the course of sample collection. We also thank Mal. Ali of the Department of Immunology, University of Maiduguri Teaching Hospital for the help he rendered during the analysis of the samples.

REFERENCES


COMPARISON OF RAPID DIAGNOSTIC TESTS AND MICROSCOPY FOR MALARIA

*Oyeniran O. A.1, Ajayi O.O.2, Afolabi A.Y.3, Oladipo E.K.4, Adepeju A.A.5

1Department of Medical Microbiology & Parasitology, Ladoke Akintola University of TechnologyTeaching Hospital, PMB 5000, Osogbo, Osun State, Nigeria. 2Department of Biological Sciences Joseph Ayo Babalola University Ikeji-Arakeji,Osun State, Nigeria; 3Department of Biomedical Science, Ladoke Akintola University of Technology, Osogbo 4Department of Pure and Applied Biology (Microbiology/ Virology Unit) Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Oyo State, Nigeria. 5Department of Chemical Pathology, Ladoke Akintola University of Technology Teaching Hospital, PMB 5000,Osogbo, Osun State, Nigeria;

*Correspondence: E-mail: lizzy05.oo@gmail.com

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 except P.ovale. 25 (12.5%) of the study population had temperature < 37.5°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 HRPii2 were 72.7% and 90.9% respectively. Both had high detection (94.7%) at parasite density ≥ 10,000 parasites/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

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 Rich Protein II et de test de diagnostic rapide (TDR) à base de kits plasmodium lactate dehydrogénase (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 parasitmique 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 parasitmiques. 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 7 months 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°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 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:

\[
\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100\%
\]

\[
\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100\% : \text{where TN represents true negative, TP true positive, FN false negative and FP false positive}
\]

**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°C was recorded in 175 cases (87.5 %) of which 83 (45%) were positive for malaria while the rest were negative. Those with temperature ≤37.5°C were 25 (12.5%) 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](image)
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). Only six samples had a *P.malariae* and *P.falciparum* with density ranging between 57 and 331 parasites/µl of blood (mean= 2.77/µl).

**Comparison Of Microscopy With Rdt5**

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 (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**

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

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Gender</th>
<th>Male</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1i20</td>
<td>Female</td>
<td>77</td>
<td>137</td>
<td>0.433</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>60</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>21i40</td>
<td>Female</td>
<td>18</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>41i60</td>
<td>Female</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>61i80</td>
<td>Female</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Female</td>
<td>111</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>89</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Clinical Symptoms</th>
<th>Number Observed</th>
<th>No. of microscopy positive (%)</th>
<th>No. of RDTS positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>148</td>
<td>65 (44)</td>
<td>54 (37)</td>
</tr>
<tr>
<td>Headache</td>
<td>121</td>
<td>38 (32)</td>
<td>31 (27)</td>
</tr>
<tr>
<td>Chills</td>
<td>113</td>
<td>47 (42)</td>
<td>39 (35)</td>
</tr>
<tr>
<td>Cough</td>
<td>36</td>
<td>5 (19)</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Tiredness</td>
<td>27</td>
<td>9 (33)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>18</td>
<td>12 (67)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>32</td>
<td>16 (50)</td>
<td>11 (34)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>31</td>
<td>12 (39)</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>10</td>
<td>2 (22)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Body pain</td>
<td>43</td>
<td>15 (35)</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>12</td>
<td>7 (58)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Sweating</td>
<td>41</td>
<td>13 (32)</td>
<td>7 (17)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Methods</th>
<th>Positive</th>
<th>% Positive</th>
<th>No Negative</th>
<th>% Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>99</td>
<td>44.5</td>
<td>101</td>
<td>55.5</td>
</tr>
<tr>
<td>HRP II</td>
<td>81</td>
<td>40.5</td>
<td>119</td>
<td>59.5</td>
</tr>
<tr>
<td>pLDH ( P. f.)</td>
<td>41</td>
<td>20.5</td>
<td>159</td>
<td>79.5</td>
</tr>
<tr>
<td>(Pf)</td>
<td>85</td>
<td>42.5</td>
<td>115</td>
<td>57.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Result/Methods</th>
<th>Microscopy</th>
<th>HRP II</th>
<th>pLDH</th>
<th>Composite Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>False positive</td>
<td>27</td>
<td>9</td>
<td>13</td>
<td>_</td>
</tr>
<tr>
<td>True Negative</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>False Negative</td>
<td>10</td>
<td>29</td>
<td>25</td>
<td>_</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Methods</th>
<th>Sensitivity(%)</th>
<th>Specificity(%)</th>
<th>Positive Predictive Value(PPV%)</th>
<th>Negative Predictive Value(NPV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>87.8</td>
<td>76.9</td>
<td>72.7</td>
<td>90.9</td>
</tr>
<tr>
<td>HRP 2</td>
<td>72.7</td>
<td>90.9</td>
<td>88.9</td>
<td>78.3</td>
</tr>
<tr>
<td>pLDH</td>
<td>84.7</td>
<td>78.3</td>
<td>84.7</td>
<td>_</td>
</tr>
</tbody>
</table>

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REFERENCES


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

Djiyep¹, A C N., Djieyep², F D., Pokam¹, B T., David³ D L., and Kamga⁴ H L F.

¹Department of Biological Sciences, Faculty of Science, Adamawa State University, Mubi, Nigeria; ²Department of Biological Sciences, Faculty of Science, Federal University of Lokoja, Nigeria; ³Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Buea, Cameroon; ⁴Department of Biological Sciences, Faculty of Science, Taraba State University, Jalingo, Nigeria; ⁵Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Bamenda, Cameroon

*Corresponding author: Henri Lucien Fouamno Kamga. Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Bamenda. Email: henrikamga2002@yahoo.fr Tel: (+237)99721972

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 of oocysts of intestinal coccidian parasites using 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.

Djiyep¹, A C N., Djieyep², F D., Pokam¹, B T., David³ D L., and Kamga⁴ 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 auto-limitées, 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 répandue du VIH/SIDA.
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+ T lymphocyte 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 diarrhea 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) and have
been implicated in conditions 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 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 are the 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 and its 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 who consented 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 parasites oocysts 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 samples were 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>
<thead>
<tr>
<th>Parastes</th>
<th>Number (%) of participant infected</th>
<th>TOTAL n=305</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=68)</td>
<td>Females (n=237)</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>32 (47.0)</td>
<td>95 (40.1)</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>23 (33.8)</td>
<td>72 (30.4)</td>
</tr>
<tr>
<td>Microsporidium species</td>
<td>2 (2.9)</td>
<td>10 (4.2)</td>
</tr>
<tr>
<td>Total</td>
<td>57 (83.8)</td>
<td>177 (74.6)</td>
</tr>
</tbody>
</table>

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 by age (P> 0.05).
TABLE 2: PREVALENCE OF COCCIDIAN PARASITES IN STUDY PARTICIPANTS ACCORDING TO AGE

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Number (%) of infected participants aged (years)</th>
<th>Total n=305</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;21 (n=30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21—50 (n=239)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;51 (n=36)</td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>10 (33.3)</td>
<td>127(41.6)</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>9 (30.0)</td>
<td>95(31.1)</td>
</tr>
<tr>
<td>Microsporidium species</td>
<td>1 (3.3)</td>
<td>12(3.9)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (66.6)</td>
<td>234(76.7)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Number (%) of patients having been treated for</th>
<th>Total n = 305</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 yr n = 85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 yrs n = 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 yrs n = 60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 yrs n = 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 yrs n = 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 yrs n = 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 yrs n = 15</td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>40(47.1)</td>
<td>127(41.6)</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>27(31.8)</td>
<td>95(31.1)</td>
</tr>
<tr>
<td>Microsporidium species</td>
<td>7(8.2)</td>
<td>12(3.9)</td>
</tr>
<tr>
<td>Total</td>
<td>74(87.0)</td>
<td>234(76.7)</td>
</tr>
</tbody>
</table>

Table 4 shows the prevalence of 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 found no 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: PREVALENCE OF PARASITES IN STUDY PARTICIPANTS ACCORDING TO CD4 COUNT

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Number (%) of infected patients with CD4 count</th>
<th>Total N= 305</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;200 n=30</td>
<td>200 - 499 n=201</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>14(46.7)</td>
<td>92(45.8)</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>11(36.7)</td>
<td>65(32.3)</td>
</tr>
<tr>
<td>Microsporidium species</td>
<td>0(0)</td>
<td>8(4.0)</td>
</tr>
<tr>
<td>Total</td>
<td>25(83.3)</td>
<td>165(82.1)</td>
</tr>
</tbody>
</table>

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 coinfection 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 Djieye conceived and designed the study, conducted the literature search, drafted the manuscript and carried out the laboratory investigations. Felicité Djieye 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


