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October 2020; Volume 21: Number 4: Pages 256 – 362

TABLE OF CONTENTS

VIEW POINT

A call: COVID-19 research funding in Africa

E. K. Oladipo., A. F. Ajayi., A. A. Oladipo., O. E. Ariyo., B. B. Oladipo., L. O. Ajayi., J. K. Oloke.....256-257

REVIEW ARTICLES

Coronaviruses: a review of their properties and diversity

A. A. Joseph., A. H. Fagbami.....258-271

Salmonella Kentucky: prevalence and challenges in Nigeria and the Africa continent

E. E. Igomu.....272-283

ORIGINAL ARTICLES

Co-infections of MERS-CoV with other respiratory viruses in Saudi Arabia

K. Al-Quthami., W. S. Al-Waneen., B. O. Al Johnyi.....284-289

Infections of implantable cardiac devices by biofilm forming bacteria in western Algeria hospitals

Z. Meziani., H. Hassaine., F. Belhachemi.....290-303

Effects of certain disinfectants and antibiotics on biofilm formation by *Staphylococcus aureus* isolated from medical devices at the University Hospital Center of Sidi Bel Abbas, Algeria

I. Kara Terki., H. Hassaine., A. Kara Terki., B. Nadira., N. Kara Terki., S. Bellifa., I. Mhamedi., M. Iachachi.....304-310

Nasal carriage of methicillin resistant *Staphylococcus aureus* among medical students of a private institution in Ilishan-Remo, Ogun State, Nigeria

T. A. Ajani., C. J. Elikwu., V. Nwadike., T. Babatunde., C. J. Anaedobe., O. Shonekan., C. C. Okangba., A. Omeonu., B. Faluyi., T. E. Thompson., E. Ebeigbe., B. G. Eze., M. A. Ajani., K. Perelade., M. Amoran., P. Okisor., T. Worancha., J. Ayoade., E. Agbeniga., C. Emmanuel., C. A. Coker311-317

Effects of highly active antiretroviral treatment on liver and renal functions of HIV-infected patients attending the day care clinic of the Bamenda Regional Hospital, Cameroon

M. Samje., E. K. J. Youego., T. W. Kefeyin., H. Lukong.....318-327

In vitro assessment of the potency of some Newcastle disease vaccine brands in Ibadan, Nigeria

A. A. Okanlawon., S. A. Ameen., R. A. Kadir., H. M. Ambali., Y. A. Baba., O. M. Azeez., A. A. Owoade.....328-332

In vivo anti-malarial activity of propranolol against experimental *Plasmodium berghei* ANKA infection in mice

O. I. Adeyemi., O. O. Ige., M. A. Akanmu., O. E. Ukponmwan.....333-339

Prevalence and factors associated with dengue fever among febrile patients attending secondary health facilities in Kano metropolis, Nigeria

M. M. Abdulaziz., A. Ibrahim., M. Ado., C. Ameh., C. Umeokonkwo., M. B. Sufyan., M. S. Balogun., S. A. Ahmed.....340-348

SHORT COMMUNICATIONS

Gastrointestinal parasites among swine bred in Edo State, Nigeria

Z. Omoruyi., I. Agbinone.....349-353

Comparative distribution of bacterial contaminants of packaged and unpackaged polyherbal products sold in Nnewi, Nigeria

C. V. Udeogu., N. R. Agbakoba., G. O. Chukwuma.....354-359

CASE REPORT

Hookworm presenting as acute febrile illness and surgical abdomen

A. M. Efunshile., C. K. Ojide., C. C. Nwangwu., G. Emelobe., C. Akpa.....360-362

**View Point****Open Access****A call: COVID-19 research funding in Africa**

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Un appel: le financement de la recherche COVID-19 en Afrique

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The World Health Organization (WHO) has made the general public to understand the transmission pattern of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is responsible for COVID-19, across different countries of the world (1). There have been reports showing increase in confirmed COVID-19 cases in low-and-middle-income countries (LMICs) such as Bangladesh, Cameroon, the Democratic Republic of Congo, India, Indonesia,

Nigeria, Mali, Gambia, Senegal, Ghana, Sudan, Tunisia and other countries of Africa. However, the scientific communities have started up various researches on COVID-19, in several areas including virus genomics and proteomics, epidemiology, pathogenesis, diagnostics, vaccines, drug discovery and therapy (including clinical trials) as well as preventive approaches to combat the pandemic. The scale and speed at which these COVID-19 researches are being

conducted in other countries is unprecedented whereas this is very low in African countries when compared to these other countries in terms of funding.

There is the urgent need for various international organizations, African health institutions, governments of African countries, private co-operations, and African philanthropists to come up with specific road maps for provision of funds for African scientists to conduct research on COVID-19 in such areas as basic research on SARS-CoV evolution and history, pathogenesis, transmission characteristics and disease modelling in the population, diagnostics, outbreak response and prevention, drug and vaccine development and clinical trials in different populations because of the heterogeneity of the African population.

According to the Frontiers Coronavirus Funding Monitor, there were little or no calls for COVID-19 research funding for African scientists either by the African governments or private institutions and organizations (2). There were calls for or from Finland, India, Belgium, Austria, Germany, USA, Denmark, Canada and Global. Still African scientists will have to compete with scientists from these developed countries, which will significantly reduce their chances of obtaining these funds.

As a result of the increasing spread of the virus and severity of the disease, COVID-19 pandemic is a serious public health emergency, and urgent steps should be taken in answering questions associated with the virus pandemic in Africa. Because of the weak economy, inadequate health infrastructures and ineffective policies of most African countries, it is imperative to strengthen the Africa research capacity by coordinated and collaborative efforts within and outside the continent, to effectively combat emerging and re-emerging virus infections. This is the basis for the clarion call for funding of African scientists to meet the peculiar need of

the continent with respect to the COVID-19 pandemic.

In many ways, COVID-19 research funding requires multilateral collaborations and approaches to scale up the win to develop the vaccines and drugs that will have more comprehensive coverage considering the science of human genetics. The GDP of African countries is projected to fall by 5-8% due to this pandemic with prediction of 90-200 billion dollars loss (3), which is going to make funding of research difficult for governments of African countries. Therefore, multinational bodies such as Bill and Melinda Gates Foundation, National Institute of Health (NIH), Medical Research Council (MRC) UK, Wellcome Trust, Canadian Institutes of Health Research (CIHR) and other public or philanthropic funders of health research as highlighted by Viergever and Hendriks (4) should come to the aid of African scientists in fighting this global pandemic through provision of grants that will help in funding of COVID-19 research enumerated above as well as in human capacity building. Africa must not be left behind in proffering solutions to the challenges of COVID-19 and to other potential pandemic infectious diseases peculiar to the continent.

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Review Article

Open Access

Coronaviruses: a review of their properties and diversity

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Abstract:

Human coronaviruses, which hitherto were causative agents of mild respiratory diseases of man, have recently become one of the most important groups of pathogens of humans the world over. In less than two decades, three members of the group, severe acute respiratory syndrome (SARS) coronavirus (CoV), Middle East respiratory syndrome (MERS)-CoV, and SARS-CoV-2, have emerged causing disease outbreaks that affected millions and claimed the lives of thousands of people. In 2017, another coronavirus, the swine acute diarrhea syndrome (SADS) coronavirus (SADS-CoV) emerged in animals killing over 24,000 piglets in China. Because of the medical and veterinary importance of coronaviruses, we carried out a review of available literature and summarized the current information on their properties and diversity. Coronaviruses are single-stranded RNA viruses with some unique characteristics such as the possession of a very large nucleic acid, high infidelity of the RNA-dependent polymerase, and high rate of mutation and recombination in the genome. They are susceptible to a number of physical agents and several chemical agents used for disinfection procedures in hospitals and laboratories. They exhibit considerable genetic and host diversity, causing diseases of gastrointestinal and respiratory system in a wide range of vertebrate hosts including humans. The high prevalence of coronaviruses in domestic and wild animals, especially bats and birds, and the propensity for their genomes to undergo mutation and recombination may lead to emergence of new coronaviruses that could pose a serious threat to human and animal health.

Keywords: coronaviruses; SARS-CoV; MERS-CoV; SARS-Cov-2; properties; diversity; review

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Coronavirus: revue de leurs propriétés et de leur diversité

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Abstrait:

Les coronavirus humains, qui étaient jusqu'à présent des agents responsables de maladies respiratoires bénignes de l'homme, sont récemment devenus l'un des groupes les plus importants d'agents pathogènes humains dans le monde entier. En moins de deux décennies, trois membres du groupe, le coronavirus (CoV) du syndrome respiratoire aigu sévère (SRAS), le syndrome respiratoire du Moyen-Orient (MERS)-CoV et le SRAS-COV-2, sont apparus, provoquant des épidémies qui ont touché des millions et des personnes. a coûté la vie à des milliers de personnes. En 2017, un autre coronavirus, le coronavirus du syndrome de la diarrhée aiguë du porc (SADS) (SADS-CoV) est apparu chez des animaux tuant plus de 24000 porcelets en Chine. En raison de l'importance médicale et vétérinaire des coronavirus, nous avons effectué une revue de la littérature disponible et résumé les informations actuelles sur leurs propriétés et leur diversité. Les coronavirus sont des virus à ARN simple brin avec certaines caractéristiques uniques telles que la possession d'un très grand acide nucléique, une infidélité élevée de la polymérase dépendante de l'ARN, et un taux élevé de mutation et de recombinaison dans le génome. Ils sont sensibles à un certain nombre d'agents physiques et à plusieurs agents chimiques utilisés pour les procédures de désinfection dans les hôpitaux et les laboratoires. Ils présentent une diversité génétique et hôte considérable, provoquant des maladies du système gastro-intestinal et respiratoire dans un large éventail d'hôtes vertébrés, y compris les humains. La forte prévalence des coronavirus

chez les animaux domestiques et sauvages, en particulier les chauves-souris et les oiseaux, et la propension de leurs génomes à subir des mutations et des recombinaisons peuvent conduire à l'émergence de nouveaux coronavirus qui pourraient constituer une menace sérieuse pour la santé humaine et animale.

Mots-clés: coronavirus; SARS-CoV; MERS-CoV; SRAS-CoV-2; Propriétés; la diversité; la revue

Introduction:

Prior to the emergence of the severe acute respiratory syndrome-coronavirus (SARS-CoV) in humans in 2002-2003, coronaviruses were not regarded as important infectious agents of humans because they generally caused mild disease in healthy and immunocompetent individuals. Human coronaviruses (HCoV)-229E and OC43 were first identified in the 1960s as causative agents of mild upper respiratory illness. About four decades later, two other human coronaviruses, HCoV-HUK1 and HCoV-NL63 were isolated from cases of bronchiolitis and pneumonia, respectively (1,2,3). Between 2002 and 2019, three coronaviruses; SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2, and one animal coronavirus; swine acute diarrhoea syndrome (SADS) coronavirus, have emerged with severe consequences (4,5,6,7).

SARS-CoV was first reported from China as the aetiological agent of a respiratory disease that affected 1755 people, out of which 774 died (4), and in 2012 MERS-CoV emerged in Saudi Arabia also causing a disease of the respiratory tract characterized by high morbidity and high mortality. In all, MERS-CoV affected over 8,000 people in 27 countries with 803 deaths (5). In, 2019, a new coronavirus, SARS-CoV-2 emerged in China initiating a pandemic that has so far sickened over three million people with more than two hundred thousand deaths in more than 190 countries (6). Although SARS-CoV disappeared after two years, sporadic cases of MERS continue to occur in some countries, and SARS-CoV-2 continues to spread worldwide (8).

New emerging coronaviruses are viruses that are harboured by wild animals, or those that arose as a result of mutation and genetic recombination. Transmission to humans may occur as a result of encroachment on the habitats of wild animals through recreational activities such as hunting, hiking and mountaineering. Furthermore, projects that include deforestation for urban development and agriculture, the use of wildlife species as companion animals, and activities around live wild animal markets may expose humans to zoonotic coronaviruses. In recent years, many researchers have been engaged in virus surveillance in wild animals leading to the discovery of many novel coronaviruses. The identification of these new viruses has been facilitated by the development

and availability of new diagnostic tests such as the polymerase chain reaction and viral genome sequencing.

The emergence of SARS-CoV-2, the causative agent of the current pandemic, has stimulated the interest and desire of the general public, scientists and healthcare givers to understand the biology of coronaviruses. The purpose of this review is to provide information on the properties of coronaviruses including their diversity, pathogenicity, and susceptibility to physical and chemical agents. Such information will lead to a better understanding of how new coronaviruses emerge, their zoonotic potential, the diseases they cause, and which types of infection control measures should be put in place to prevent their spread.

Methodology:

This is a review of published articles and textbooks, printed and online, accessed through matched search using Google and Bing search engines to peruse articles indexed in Google Scholar, PubMed, Scopus, Index Medicus and AJOL. The keywords used for the online search included 'coronaviruses', 'properties', 'SARS CoV', 'MERS', 'SARS CoV2' singly and combined. The review of relevant materials was done over a period of 8 weeks between February and April 2020. Documents and materials were reviewed and those that met the criteria in quality and relevance to the topic were selected while others were excluded. In total, four (4) print textbooks, seventy-seven (77) original articles and one (1) committee report were utilized for the review.

Classification of coronaviruses

Coronaviruses belong to the family coronaviridae which is divided into two subfamilies; orthocoronavirinae and torovirinae. Based on phylogenetic analysis and serological properties, the orthocoronavirinae is divided into four genera; genus *alphacoronavirus*, genus *betacoronavirus*, genus *deltacoronavirus*, and genus *gammacoronavirus*. The first two genera contain only mammalian coronaviruses while all avian coronaviruses are in the last two genera (9). A few animal coronaviruses have been placed along with the avian species in the genus *deltacoronavirus*.

Major properties of coronaviruses

Coronaviruses are spherical in shape with a diameter of 80-160 nm and an envelope that is covered by widely spaced club or petal-shaped projections, 20 nm in diameter (Fig 1).

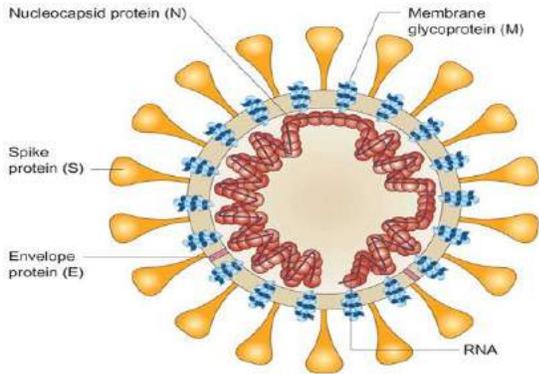


Fig 1: schematic diagram of electron microscopic structure of coronavirus
Source: Peiris, Joseph S. 2005⁸¹

The arrangement of the projections gives the envelope the characteristic crown-like appearance from which the family name 'corona' was derived (10). They have a single-stranded, non-segmented RNA with positive sense, 27-32 kb in size, and of all RNA genomes, the coronavirus genome is the largest.

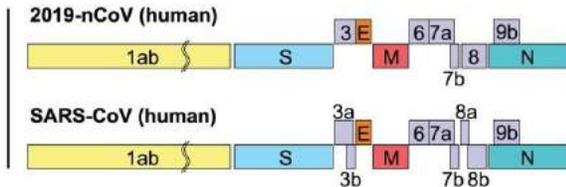


Fig 2: Genomic Organization of SARS-CoV and SARS-CoV-2 (2019-nCoV)
yellow box = open reading frame (orf) 1ab; blue box = spike protein (S); purple boxes = open reading frame (orf) 3, 6, 7a, 7b, 8 and 9b (accessory proteins); orange box = envelop (E) protein; red box = membrane (M) protein; cyan box = nucleocapsid (N) protein. Source: Chan JF et al. 2020⁸².

The RNA is infectious, capped, and polyadenylated (Fig 2), and can serve as mRNA. It encodes four structural proteins, membrane (M) protein, spike protein (S), envelope protein (E), and viral nucleoprotein (N). Some beta-coronaviruses have a fifth structural glycoprotein, the haemagglutinin-esterase (HE), on the surface of the virion that is genetically related to a similar protein, the haemagglutinin-esterase fusion protein of influenza C virus (11). The S protein interacts with specific receptors on

the host cell to facilitate attachment, viral entry, and induction of immune response. The genome also encodes nonstructural proteins which include two replicase polyproteins, ORF1a and ORF1b, and between one or more accessory proteins that perform important functions in coronavirus replication and in vivo pathogenesis

Replication of coronavirus

The replication of coronaviruses is complex and has been previously reviewed by several authors (12,13). To initiate replication, coronavirus attaches to a receptor on the host cell with the aid of the glycoprotein S on the viral envelope, and HE in some betacoronaviruses. The receptor for the human coronavirus HCoV-229 E is aminopeptidase N (APN), whereas, it is angiotensin-converting enzyme 2 (ACE-2) for SARS-CoV and SARS-CoV-2, and dipeptyl peptidase 4 (DPP4) for MERS-CoV. Viral entry is facilitated by the fusion of the S protein with the cell membrane, following which the viral genome RNA is uncoated.

The genomic RNA is used as template to translate a polyprotein that is employed to generate an RNA-dependent RNA polymerase, with which a full-length negative strand template is synthesized. Subsequently, the negative strand template is used to generate a nested set of 5-7 subgenomic mRNAs by a discontinuous transcription mechanism (13), and a full-length genomic RNA. These subgenomic mRNA possess common 5' leader and 3' terminal sequences. Only the unique sequences at the 5' end of the mRNAs are translated to form non-structural proteins and structural proteins including M, E, S and N proteins, and in some viruses, the HE protein. Newly synthesized genomic RNA molecules and the nucleoprotein assemble in the cytoplasm to form the helical nucleocapsids, which bud through the membranes of the endoplasmic reticulum at the area that contains viral glycoproteins. Mature virions may be transported in vesicles, and released by fusion of the vesicles with plasma membrane (12,13).

Effects of physical and chemical agents on coronaviruses

Endemic human coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1) as well as SARS-CoV and MERS-CoV are susceptible to a number of physical agents including pH, temperature, and relative humidity. They are rapidly inactivated by very acidic and alkaline pH, but are more stable at pH 6-6.5 (14,15). Exposure of SARS-CoV to temperature of 56°C for 30 minutes inactivates it, however,

in the presence of protein, a higher temperature is required to achieve virus inactivation. Dried virus on smooth surfaces remains infectious for over 5 days at temperatures of 22°C-25°C and relative humidity of 40-50%, but virus infectivity was rapidly lost at higher temperatures of up to 38°C and relative humidity of >95% (15). Chan et al., (15) therefore suggested that the stability of SARS-CoV at temperatures similar to air-conditioned room temperatures and low humidity may facilitate cross contamination and virus transmission at home, workplace and public institutions. They further suggested that corona viruses may be inactivated quickly in tropical areas with high ambient temperatures and relative humidity, thereby reducing virus transmission and spread in communities.

Coronaviruses are also susceptible to treatment with several chemical agents and disinfectants as well as antiseptics. As enveloped viruses, they are readily inactivated by ether and chloroform, as well as other chemical agents such as ethanol, propanol, glutaraldehyde, and formaldehyde. According to Kampf (16), human coronaviruses such as SARS-CoV, MERS-CoV and endemic human coronaviruses can persist on inanimate objects like metal, glass, or plastic for up to 9 days, but can be inactivated easily by surface disinfection procedures with 62-71% ethanol, 0.5% hydrogen peroxide, or 0.1% hypochlorite within one minute. In another study, it was found that ethanol and isopropanol as well as WHO recommended handrub formulations efficiently inactivated SARS-CoV and SARS-CoV-2 (17). Sattar et al., (18) assessed the virucidal efficacy of several antiseptics and found that hexamidine, chlorhexidine and cetrimide were ineffective against HCoV 229E, but if alcohol was added, both chlorhexidine and cetrimide acquired virucidal properties.

Growth of coronaviruses in tissue culture

The identification of SARS CoV, SARS-CoV-2, and MERS-CoV, and the need to characterize and develop vaccines against them demand that they be adapted to cell cultures so that adequate quantities of infective virus could be available for further studies. Coronaviruses are difficult to propagate in cell cultures and human coronaviruses have traditionally been grown in human organ and cell cultures (12). HCoV-229E grows readily in primary or secondary human embryonic cultures and human fibroblast cell lines, much better than HCoV-OC43. The most sensitive cell line for the isolation of these viruses from clinical specimens

seems to be the diploid intestinal cell line, MA-177 (19).

Studies on the newer coronaviruses showed that clinical isolates of SARS-CoV replicated efficiently in a variety of cell cultures such as VeroE6 and LLC-MK2 (monkey kidney), HEK-293T (human kidney), RK-13 (rabbit kidney), Huh (human hepatocellular carcinoma), and human peripheral blood mononuclear cells (PBMCs) (20,21). Similarly, MERS-CoV grew in monkey kidney cell cultures and Huh cell line, but did not grow in a variety of other cell lines (22). Recent studies have shown that SARS-CoV-2 can be propagated in the same cell cultures that are useful for cultivating SARS-CoV, and that the primary human airway epithelial cell culture is the most sensitive for growing the virus (23).

Susceptibility of laboratory animals to coronavirus infection

Laboratory animal models are often used for experimentation towards vaccine or drug development. Such animal models should be susceptible to infection and produce the type of clinical manifestations observed in humans (24). Efforts were made by researchers to develop animal models for SARS-CoV and MERS-CoV, and similar efforts are currently in progress for SARS-CoV-2. In experimental transmission studies, SARS-CoV produced clinical disease in mice, hamsters, ferrets, and sub-human primates, and except for old mice, none developed severe disease (24,25). Similar studies carried out with MERS-CoV have largely been unsuccessful, but subhuman primates and transgenic mice were susceptible to infection (25). Shi et al., in their own studies showed that although SARS-CoV-2 replicated poorly in dogs, pigs, chicken and ducks, the virus grew well in ferrets and cats (26). Other studies have documented severe disease in hamsters infected with SARS-CoV-2 that is similar to the upper and lower respiratory tract disease in humans, and mild disease and asymptomatic infection in rhesus monkeys and mice, respectively (27,28).

Genetics of coronavirus

Two phenomena, mutation and recombination, account for genetic diversity among viruses. By definition, mutation is a change in the viral genome which can occur by substitution, insertion, and deletion of bases, as a result of the high error rates and lack of an effective proof-reading mechanism in the viral polymerase (12). Coronaviruses exhibit a high frequency of mutation during each replication

cycle, occurring along the whole length of the genome, and affecting the *S*, *M*, *E*, and the *N* genes, which may have little or no effect on the virus, or in some cases, cause major changes in viral characteristics. These changes can be demonstrated not only experimentally, but also in natural infections. Zhao et al., (29) experimentally showed that accumulations of mutations in the *S* gene and the accessory gene *5a* accounts for loss of pathogenicity of the infectious bronchitis virus (IBV), a coronavirus of chicken.

The porcine respiratory coronavirus, the causative agent of a mild respiratory disease in pigs evolved naturally as an *S* gene deletion mutant of the transmissible gastroenteritis virus (TGEV) of pigs. The mutation changed the tissue tropism of the virus for the gastrointestinal tract to the respiratory tract (30). Another coronavirus that emerged naturally as a result of mutation is the feline infectious peritonitis virus (FIPV), a mutant of feline enteritis coronavirus (FECV), which unlike its parent coronavirus, FECV, that causes benign enteritis, is very virulent, causing a severe and fatal disease in cats (31). As coronavirus infects new hosts and spreads from person to person, it mutates; and such mutations can be used to determine the source of the virus and monitor its spread. Ruan et al., (32) studied 14 isolates of SARS-CoV and showed that common mutations are associated with geographical origins of infection. Such mutations are now being used by scientists to monitor the trajectory of the current SARS-CoV-2 pandemic as it spreads globally.

Recombination occurs often in coronaviruses and is an important mechanism by which viruses generate genetic diversity (33,34), contributing to the emergence and evolution of different coronavirus genotypes and species. Recombination is the exchange of nucleic acid fragments or segments between two different, but closely related viruses, resulting in the production of certain progeny viruses with genes from both parental viruses. It often leads to increase or decrease in virulence, altered host range, and appearance of new viral antigens. Unlike the other non-segmented RNA viruses which exhibit lower recombination rates, the frequency of recombination in coronaviruses is high, and is probably due to the unique mechanism of replication which involves discontinuous transcription and polymerase jumping (33,34).

The biological significance of recombination was recently demonstrated when Jackwood et al., (35) provided an evidence that recombination can directly lead to the emergence of new coronaviruses. He and his co-workers showed that the turkey coronavirus

(TCoV) emerged from a recombination event that replaced the *S* gene of IBV with an *S* gene from unidentified source, accompanied by a change in tissue tropism. The recombinant virus acquired enteropathogenic property causing diarrhea in turkeys, whereas, IBV, one of the parent viruses, was pneumotropic. Similarly, Boniotti et al., (36) described the identification of swine enteric coronavirus (SeCoV) which emerged as result of a recombination between TGE and porcine epidemic diarrhea (PED) viruses, and whose pathogenicity for pigs is much less than that of the parental viruses. Recombination is quite common among coronaviruses of wild animals. Lau et al., (37) showed that each of the three deltacoronaviruses isolated in the UAE; falcon coronavirus HKU 27, houbara bustard coronavirus HKU28, and pigeon coronavirus HKU29, originated from recombination between two avian coronaviruses.

Diversity of coronaviruses

Coronaviruses infect a diverse range of vertebrate hosts including humans, animals, and birds causing diseases of gastrointestinal and respiratory system or both, and some may affect other organ systems including the central nervous system and the renal system, or cause subclinical infection. Within each host specie, many genetically diverse coronaviruses have been identified, mostly among bats and birds.

Human coronaviruses

The coronaviruses of medical importance are shown in Table 1. They include four human coronaviruses; HCoV [HCoV-229E, HCoV-OC43, HCoV-Hong Kong University (HKU) 1 and HCoV-NL63], SARS-CoV, MERS-CoV, and SARS-CoV-2. Human coronavirus 229E and OC43 were first isolated in the 1960s from adult patients with common colds (38,39), and in 2004, HCoV-NL63, also known as New Haven coronavirus, was identified in an infant with bronchiolitis in the Netherlands (40). The HCoV-HKU 1 was discovered in 2005 in an elderly man, a Hong Kong resident, who developed pneumonia following a visit to China (41). In general, all these HCOVs, except HKU1, produced mild respiratory disease in infected patients.

SARS-CoV, the causative agent of an atypical pneumonia characterized by a high morbidity and high mortality, was first described in 2002 in China (4). It has been reported in several Asian countries, Europe, and North America, but most cases have occurred in China, Taiwan, Hong Kong, and Canada. The virus has the properties of other coronaviruses; however,

its genomic sequence is different from that of the HCoV, although it enters the host cell through ACE-2, like the HCoV NL63 and SARS-CoV-2 (30). In 2012, MERS coronavirus, the causative agent of a severe respiratory illness characterized by fever, shortness of breath, pneumonia, and high mortality, was first identified in Saudi Arabia (5). Seven years later in December 2019, a novel coronavirus, the SARS-CoV-2 was identified in patients with pneumonia in Wuhan, Hubei Province, China. The disease caused by this virus, named COVID-19, has affected over 80,000 people with more than 3,000 deaths in China, and over three million cases and 200,000 deaths worldwide (6), SARS-CoV 2 shares about 70% to 80% of its genome with SARS-CoV, but it is most genetically related (96% similarity) to a horse shoe bat coronavirus. It uses the same ACE-2 receptor to enter the host cells like SARS-CoV, but has a longer S protein and a different binding region (42,43,44).

All the seven coronaviruses that have caused disease in humans are believed to have

a zoonotic origin, transmitted to man from bats or other animals through intermediate hosts that include domestic and wild animals (44-51) (Table 2). Corman et al., (48) proposed the bat origin of HCoV-229E when they detected a bat coronavirus in Ghanaian *Hipposideros caffer ruber* bats which shared 92% nucleotide sequence identity with HCoV-229E. Similarly, Donaldson et al., (49) and Tao et al., (50) isolated bat coronaviruses that have a close relationship with HCoV-NL63, also supporting the theory of bat origin of HCoV-NL63. Although the probable intermediate host between the bat NL63-related coronavirus and HCoV-NL63 is not known, Crossley et al., (51) identified the alpaca as the probable intermediate host between the bat HCoV-229E-related virus and HCoV-229E. But unlike HCoV-229E and HCoV-NL63 which have ancestral bat origin, HCoV-OC43 is believed to have evolved from ancestral BCoV strains that crossed the interspecies barrier from cattle and established infection in humans (52,53).

Table 1: Coronaviruses affecting humans and their classification

Coronavirus	Genus	Year of Original Isolation	Disease
Human coronavirus 229 E	Alpha	1960s	Mild respiratory disease
Human coronavirus NL63	Alpha	2005	Mild respiratory disease
Human coronavirus OC43	Beta	1960s	Mild respiratory disease
Human coronavirus HKU 1	Beta	2004	Pneumonia
Severe acute respiratory syndrome CoV	Beta	2002	Severe acute respiratory disease
Middle East respiratory syndrome CoV	Beta	2012	Severe acute respiratory disease
Severe acute respiratory syndrome CoV-2	Beta	2019	Severe acute respiratory disease

Table 2: Ancestral and intermediate hosts of coronaviruses pathogenic to humans

Host	Coronavirus	Ancestral/Reservoir Host	Probable Intermediate Host
Humans	Human coronavirus NL63	Bat (<i>Perimyotis subflavus</i> , <i>Triaenops afer</i>)	?
	Human coronavirus 229 E	Bat (<i>Hipposideros caffer ruber</i>)	Alpaca
	Human coronavirus OC43	Mouse	Cattle
	Human coronavirus HKU 1	Mouse	?
	Severe acute respiratory syndrome-CoV	Bat (<i>Rhinolophus</i> spp)	Palm civet cat Raccoon
	Middle East Acute respiratory syndrome-CoV	Bat (<i>Taphozous perforatus</i> , <i>Rhinopoma hardwickii</i> and <i>Pipistrellus kuhlii</i>)	Dromedary camel
	Severe Acute respiratory syndrome-CoV-2	Bat (Horseshoe bat)	? Mink ? Pangolin

Following the emergence of SARS-CoV in 2002, SARS-related viruses in Rhinolophus horseshoe bats and palm civet cats were discovered by scientists. Based on these discoveries, the scientists concluded that SARS-CoV originated from bats and that palm civet cats were the intermediate hosts from which humans acquired infection (46,54). MERS-CoV also emerged in 2012 and subsequent studies led to the isolation of MERS-related CoVs from several species of bats as well as dromedary camels (45,54). Scientists therefore suggested that the natural hosts of MERS-CoV were bats and the intermediate hosts were camels (45,46,54). Similarly, SARS-CoV-2 emerged following virus transmission between a horseshoe bat and an intermediate host which is probably a mink or pangolin or other yet to be identified animals (44,47).

Coronaviruses of domestic animals and birds

Coronaviruses of domestic animals and birds are found in the four genera of the sub-family coronavirinae; alphacoronavirus, betacoronavirus, deltacoronavirus and gamma-

coronavirus. All mammalian coronaviruses except porcine deltacoronavirus, are found in the genus alphacoronavirus and betacoronavirus, whereas, all avian coronaviruses are in the delta and gammacoronavirus. Animal and avian coronaviruses predominantly cause enteric and pulmonary, as well as hepatic, renal and neurological syndromes (55), affecting swine more than any other domestic animal species. In all, there are fourteen coronaviruses of veterinary importance including seven porcine, two each of feline and avian, and one each of bovine, canine, and equine coronaviruses (Table 3).

Porcine coronaviruses include the transmissible gastroenteritis virus (TGEV), porcine haemagglutinating encephalomyelitis (PHE), porcine epidemic diarrhea virus (PEDV), porcine respiratory coronavirus (PRCoV), porcine delta coronavirus (PDCoV), swine acute diarrhea syndrome coronavirus (SADS-CoV), and swine enteric coronavirus (SeCoV). All are primarily enteropathogenic, except the PRCoV which is a respiratory pathogen, and all have only one serotype.

Table 3: Important coronaviruses of domestic animals and birds

Coronaviruses	Genus	Host	Disease
Transmissible gastroenteritis virus	Alpha	Swine	Gastroenteritis
Porcine epidemic diarrhea CoV	Alpha	Swine	Gastroenteritis
Porcine respiratory CoV	Alpha	Swine	Respiratory disease
Swine acute diarrhea syndrome CoV	Alpha	Swine	Diarrhoea
Swine enteric coronavirus	Alpha	Swine	Diarrhoea
Feline enteritis coronavirus	Alpha	Cats	Enteritis
Feline infective peritonitis virus	Alpha	Cats	Peritonitis, pneumonia, meningoencephalitis, wasting syndrome
Canine coronavirus	Alpha	Dogs	Enteritis
Canine respiratory CoV	Alpha	Dogs	Respiratory disease
Bovine coronavirus	Beta	Cattle	Diarrhoea
Equine coronavirus	Beta	Horse	Colic and diarrhoea
Porcine haemagglutinating encephalomyelitis virus	Beta	Swine	Vomiting, wasting, and encephalomyelitis
Porcine deltacoronavirus	Delta	Swine	Diarrhoea
Infectious bronchitis virus	Gamma	Chicken	Tracheobronchitis, nephritis
Turkey coronavirus	Gamma	Turkey	Bluecomb, Enteritis

Transmissible gastroenteritis virus (TGEV) was identified in 1946 in the United States (56) as the causative agent of a disease of pigs characterized by vomiting, profuse diarrhea, weight loss, and dehydration, and 100% mortality among newborn pigs. Several years after the identification of TGE, another disease of pigs, the porcine haemagglutinating encephalomyelitis (PHE) was reported in Canada, but its viral aetiological agent was not isolated until 1962 (57). Recent genetic studies have shown PHE virus to be closely related to HCoV-OC43 and BCoV (53, 58). Porcine epidemic diarrhea virus (PEDV), a coronavirus that was first described in England in 1971, is similar to TGEV, causing watery diarrhea and vomiting in piglets which often leads to dehydration and death (58,59). The virus was genetically divided into 2 groups: GI and GII, both of which can be further subdivided into subgroups Ia and Ib, and subgroups IIa and IIb, respectively. Members of the subgroups exhibit marked differences in virulence, and sequence analysis of the nucleocapsid protein gene has shown that PEDV is genetically related to HCoV-229E and TGEV (60, 61).

In 2009, PDCoV was first reported in China, but its aetiologic role was not determined until 2014, when it caused diarrhea in pigs in the USA (62). The mechanism of the evolution of the virus is not well understood, but based on the widespread prevalence in song birds and data from genomic studies, it probably originated from an ancestral avian PDCoV (62). The virus utilizes a conserved region of the APN gene to infect cells derived from humans and other species (63). Whether or not it will be able to replicate in human cells and cause disease, is yet to be determined. The detection of PDCoV RNA in fecal samples of wild birds, Chinese ferret badgers and leopard cats, and the susceptibility of other animal species to experimental infection, coupled with the presence of APN receptor in many animal species including humans suggest that PDCoV has a broad host range and may have a zoonotic potential (62,63,64).

Swine acute diarrhea syndrome (SADS) CoV is the causative agent of a fatal swine acute diarrhea, a disease that primarily affects newborn pigs, characterized by severe watery diarrhea, dehydration and death. The virus is a HKU2-like coronavirus isolated in 2016 in China from horseshoe bats, *Rhinolophus* spp, (7). Recently, Boniotti et al., (36), also reported the identification of another coronavirus, the swine enteric coronavirus (SeCoV), a TGEV/PEDV recombinant virus, the causative agent of watery diarrhea in piglets. It is noteworthy that

this recombinant virus is less virulent than TGEV and PEDV, its parental viruses, producing a much lower mortality rate in piglets (65). Before the appearance of PDCoV and SADS-CoV, the porcine respiratory coronavirus (PRCoV) emerged in Belgium in 1984 as a deletion mutant of TGEV and a causative agent of a mild respiratory disease of piglets (30). The rapid spread of PRCoV to many pig farms in many countries led to widespread immunity to the virus as well as TGEV among pigs, thus reducing the veterinary importance of TGEV worldwide (65,66).

Several coronaviruses cause diseases in companion animals, cats and dogs. Feline coronavirus is a common enteric pathogen of cats existing as two biotypes: feline enteritis coronavirus (FECV) and the feline infectious peritonitis virus (FIPV) (66,67). FECV infection is generally asymptomatic, but in young kittens, mild transient diarrhea occurs, which may sometimes be accompanied by vomiting. FIPV, an FECV mutant, is the aetiological agent of an immune-mediated, progressive, and fatal disease occurring often in cats less than two years of age. It may present as the effusive, also known as the 'wet' form in immunocompromised cats with defective cell mediated immunity, or as the non-effusive, otherwise called the 'dry' form in cats with partial cell mediated immunity. The effusive form is characterized by leakage of protein-rich fluid from the blood vessels into the abdominal cavity producing abdominal distension. Cats with the non-effusive form develop pyogranulomatous or granulomatous lesions in several tissues (66).

Canine coronavirus (CCoV) was first identified in 1971 as a causative agent of mild diarrhea in dogs. The disease often occurs in association with canine parvovirus. It is closely related to TGEV, ferret CoV and feline CoV. Several strains of the virus now exist as a result of mutations in the viral genome (66,67). There are two serotypes of the virus with different biological properties. Serotype I canine coronaviruses grow poorly in tissue culture and have an ill-defined receptor, whereas, serotype II canine coronaviruses grow well in culture, using the APN receptor (67,68). Another coronavirus of dogs is the canine respiratory coronavirus (CRCoV) which was first identified in 2003 as the causative agent of canine infectious respiratory disease of dogs, also known as 'kernel cough' (66,69). The virus is genetically different from the enteric canine coronavirus and belongs to the genus betacoronavirus, unlike the enteric CCoV which is an alphacoronavirus. It is however, genetically similar to the bovine coronavirus (BCoV) and the HCoV-OC43; and possesses a haemagglutinin-esterase (HE) gene

which is absent from enteric CCoV (69). Clinically, the virus infection often presents as a respiratory disease characterized by respiratory distress, pneumonia, anorexia and sometimes, death (66).

Bovine coronavirus (BCoV) causes diseases of respiratory tract in cattle, and produces gastroenteritis or winter dysentery in lactating cows, and shipping fever pneumonia in feedlot cattle (66). The virus was first described as a cause of diarrhea in calves in the USA in 1973, but has now been documented worldwide. Phylogenetic analysis of BCoV has shown that it is closely related to HCoV-OC43, and coronaviruses identified in dogs with respiratory disease, other ruminants with enteric and/or respiratory disease including water buffalo, sheep, goats, camels, llama and alpaca, and wild ruminants such as deer, wild cattle, antelopes, giraffe and wild goats (70). Following biological, antigenic and genetic analyses, it was proposed that these bovine-like CoVs be regarded as host range variants of BCoV rather than distinct virus species (70).

Equine coronavirus is a betacoronavirus causing a disease of horses characterized by fever, anorexia, colic and occasional diarrhea after an incubation period of 2-4 days (66). The disease usually affects horses over two years of age with a morbidity that ranges from 10% to 83%, and no mortality in most cases. The virus was first isolated from the faeces of a diarrheic foal in 1999 in the USA (71), and was found to be very closely related phylogenetically to BCoV, HCoV-OC43, and PHEV (72). Two avian coronaviruses exist; infectious bronchitis virus (IBV) and turkey coronavirus (TCoV). IBV causes a highly infectious upper respiratory tract disease in commercial poultry. Infected chicken presents with watery eyes, mucus in the nares and trachea, gasping breath, cough and tracheal rales. Among layers, there can be a decrease in egg production and egg quality (55,66). Some strains can cause interstitial nephritis, pneumonia due to secondary bacterial infection, airsacculitis, and peritonitis. Morbidity is almost 100%, but mortality is low and variable, but could range from 14% to 82% depending on age of the birds, virus strain and whether the birds are coinfecting or superinfected by other pathogens (66).

Antigenically distinct serotypes of IBV appear from time to time as a result of mutation and recombination (55). IBV and IB-like coronaviruses have been found in other domestic or captive birds including pigeons, ducks, pheasants, guinea fowls and geese that were reared in the vicinity of chickens (55,66,74). Turkey

coronavirus was first identified in the United States in 1973 as the causative agent of bluecomb, a disease of turkeys that was first described in 1951 (73). The virus emerged from a recombination event that replaced the S gene of IBV with an S gene from unidentified source (55).

Coronaviruses of laboratory animals

Four coronaviruses have been identified in laboratory animals; mouse hepatitis virus (MHV), rat coronavirus (also known as sialodacryoadenitis coronavirus), guinea pig coronavirus (causing diarrhea and enteritis), and rabbit coronaviruses (66). Mouse hepatitis virus has two strains; enterotropic and polytropic strains; the former is very common in mouse colonies. Enterotropic strains of MHV are highly contagious, and often cause severe disease in mouse colonies with mortality rate that may be about 100% among infant mice. Polytropic strains of MHV are less contagious and general cause inapparent infections, although they may occasionally produce neurological disease.

Rat coronavirus (RCoV), a virus that is closely related to MHV, is the causative agent of epizootics of respiratory disease in laboratory rat colonies. There are two prototype strains of the virus; sialodacryoadenitis virus (SDAV) and Parker's rabbit coronavirus (RCoV-P) (75,76,77). Both strains infect the respiratory tract but in addition, SDAV can also infect the eye, salivary glands and lacrimal glands. The disease occurs in rats of all ages, but young rats are the most susceptible, developing lower respiratory tract infection which often leads to interstitial pneumonia. Clinical disease in older rats is characterized by nasal and ocular discharge, cervical swelling, photophobia, keratitis, and dyspnea (66,77). Rabbit coronaviruses have not been properly characterized, but have been associated with enteric disease that is characterized by malabsorption and diarrhea.

Coronaviruses of wild animals

Many coronaviruses of wild mammals and birds have been identified and are found in all the four genera of the family coronaviridae. However, some of these are presumptive new coronaviruses, yet to be approved by the International Committee on Taxonomy of Viruses (ICTV) (78). Of all species of wildlife, the vast majority of coronaviruses found to date were detected in bats and those approved by the ICTV have been placed in two genera; genus alphacoronavirus and betacoronavirus (Table 4). Bat coronaviruses genetically related to coronaviruses of medical importance include the NL-63-related bat CoV, HCoV-229E-related bat

Table 4: Bat coronaviruses

Genus	Virus	Human/ Animal virus relative
Alphacoronavirus	Bat coronavirus HKU10	
	Bat coronavirus CDPHE15	
	<i>Rhinolophus ferrumequinum</i> alpha-coronavirus HuB-2013	
	<i>Myotis ricketti</i> alpha CoV Sax 2011	
	<i>Nyctalus relutinus</i> alphaCoV SC-2013	
	NL-63-related bat coronavirus strain	Human CoV NL 63
	HCoV-229E bat coronavirus	Human CoV 229E
	Miniopterus bat coronavirus 1	
	Miniopterus bat coronavirus HKU 8	
	Rhinolophus bat coronavirus HKU 2	Swine acute diarrhea syndrome virus (SADSV)
Betacoronavirus	Scotophilus bat coronavirus 512	Porcine epidemic diarrhea virus (PEDV)
	Pipistrellus bat coronavirus HKU 5	MERS-CoV
	Rousettus bat coronavirus HKU 9	
	SARS-related rhinolophus bat CoV	SARS-CoV
	Tylosycteris bat CoV	MERS-CoV

CoV, SARS-related CoV, and MERS-like bat CoV. Others (Table 4) are genetically related to causative agents of diseases of domestic animals such as the *Rhinolophus* bat coronavirus HKU 2 which is similar to the aetiological agent of SADS (7), and the *Scotophilus* bat coronavirus 512 that was found to be very closely related to the PEDV.

Alphacoronaviruses also cause disease in other wild animals such as ferrets, mink, foxes and wild dogs (Table 5). First isolated in 1993, ferret coronavirus, is the causative agent of catarrhal enteritis of ferrets, as well as a systemic disease that resembles the dry form of feline infective peritonitis (66,67,79). Similar coronaviruses have been found in wild canines including foxes, raccoon dogs, wild dogs and mink (66,80). Betacoronaviruses also exist in wild mammals (Table 5), but unlike the alphacoronaviruses, they do not cause disease in their hosts, except the bovine-like coronaviruses that produce gastrointestinal disease in their definitive hosts that include deer, water bucks, giraffe, antelopes and others (66,70).

Many deltacoronaviruses have also been isolated from wildlife in different geographical regions of the world; those approved by the ICTV belong to seven avian coronavirus species

and one porcine coronavirus specie (Table 5). However, other deltacoronaviruses have been identified in wild birds by Hong Kong University (HKU) including sparrow coronavirus HKU 19, falcon coronavirus HKU27, Houbara bustard coronavirus HKU 28, pigeon coronavirus HKU29 and quail coronavirus HKU30 (37). These viruses probably cause subclinical disease in their definitive hosts as only the bulbul coronavirus has been associated with respiratory disease. The gammacoronaviruses of wild animals are infectious bronchitis (IB)-like coronaviruses and Beluga whale coronavirus (Table 5).

IB-like coronaviruses detected in birds include coronaviruses of pheasants, gulls, partridges, quails, and peafowls, however, they differ in the clinical manifestations of infection in their definitive hosts. For example, while pheasants develop serious disease characterized by enteritis with renal involvement, other birds such as parrots and partridges develop asymptomatic infection (55,81). Gammacoronaviruses can be transmitted from wild birds to poultry and from poultry to wild birds, and because of their ability to fly long distances they are capable of disseminating coronaviruses from one continent to another (55,81).

Table 5: Major coronaviruses of non-bat wild mammals and birds

Coronavirus	Genus	Host	Disease
Ferret coronavirus	Alpha	Ferret	Enteritis, systemic disease
Mink coronavirus 1	Alpha	Mink	Enteritis
Canine-like coronavirus	Alpha	Wild dogs, raccoon dog, foxes	Enteritis
Hedgehog coronavirus HKU 31	Beta	Hedgehog	
Murine coronavirus	Beta	Rodent	
SARS-related palm civet cat CoV	Beta	Civet cat	
SARS-related raccoon dog CoV	Beta	Raccoon dog	
Bovine coronavirus-like viruses	Beta	Deer, giraffe, antelopes, wild dogs Asian leopard cat	Enteritis
Asian leopard cat coronavirus	Beta		
Chinese ferret badger coronavirus	Beta	Chinese ferret badger	
Bulbul coronavirus HKU 11	Delta	Bird	Respiratory disease
Coronavirus HKU 15	Delta	Pig	
Munia coronavirus HKU 13	Delta	Bird	
Night heron coronavirus HKU 19	Delta	Bird	
Thrush coronavirus HKU 12	Delta	Bird	
White-eye coronavirus HKU 16	Delta	Bird	
Magpie robin Co HKU 18	Delta	Bird	
Wigeon coronavirus HKU 20	Delta	Bird	
Common moorhen Co HKU 21	Delta	Bird	
Avian coronaviruses	Gamma	Bird (pheasant, peafowl, partridges quail and parrots)	Respiratory disease, nephritis in pheasants, enteritis in quail
Beluga whale coronavirus SW1	Gamma	Whale	Respiratory disease

Conclusions:

Coronaviruses have caused three major epidemics in the human population that affected more than three million people with several thousand deaths worldwide, thus making them one of the most dangerous groups of human pathogens ever known. As respiratory viruses that remain viable in the environment for several days at temperatures similar to room temperature (22°C - 25°C) and relative humidity of 40-50%, they are readily transmissible from person to person via contaminated humans and inanimate objects such as door handles, elevator buttons, and surfaces. However, most coronaviruses including SARS-CoV, MERS-CoV and SARS-CoV-2 are susceptible to several common disinfectants that can be used to disinfect homes, workplace, laboratories, and

hospitals, and prevent virus transmission. Coronaviruses are difficult to propagate *in vitro*, but progress has been made in growing SARS-CoV and SARS-CoV-2 in tissue culture as well as in laboratory animals. The successful adaptation of SARS-CoV-2 to several cell cultures and the susceptibility of laboratory animals to its experimental infection will promote further *in vitro* and *in vivo* studies on the virus that could facilitate vaccine, drug, and diagnostic assay development.

Several coronaviruses are circulating in the human population, and a very large number of them exist in domestic and wild animals. Although many domestic animal coronaviruses exist, none of them has so far posed a threat to human health. In contrast, wild animals harbour

a variety of coronaviruses, some of which have been associated with important human and animal diseases. Given the presence of a large number of coronaviruses in wild animals, the possibility of coinfection of these animals by more than one coronavirus permitting interaction between their genomes, their propensity to undergo mutation and recombination, and their ability to jump interspecies barrier, it is likely that more new coronaviruses will emerge in future that will pose a major threat to human and domestic animal health. As long as humans continue to interface with wild animals in their natural habitats, zoos, and markets or use them as pets, more zoonotic transmissions of animal coronaviruses are likely to occur which could lead to future epidemics, or even pandemics.

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**Review Article****Open Access****Salmonella Kentucky: prevalence and challenges in Nigeria and the Africa continent**

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Salmonella Kentucky is ubiquitous in most African countries and the multidrug resistant (MDR) strains remain underreported across the continent. In Nigeria, poverty, inter country livestock trades, nomadic system of cattle production, indiscriminate use of antibiotics and prevalent immuno-compromising diseases such as human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) and tuberculosis are factors that have enabled ease of transmission and complications of *S. Kentucky* infections. In the present decade, *S. Kentucky* is reported to be the most prevalent serovar associated with poultry in Nigeria, but very few reports underline the risk associated with consumption of poultry and acquisition of MDR *S. Kentucky* strains. The Nigerian poultry is one of the most commercialized subsectors of Nigerian agriculture, therefore, the presence of *S. Kentucky* especially strains carrying broad spectrum antimicrobial resistance pose a great risk to public health. The lack of proper monitoring, surveillance, isolation and control of the multidrug resistant *S. Kentucky* will remain a challenge to the export potential of the Nigerian poultry subsector and livestock in general. As a nation, modalities and actions against the smuggling of poultry products, indiscriminate use of antibiotics and nomadic system for the production of dairy and beef that promotes spread of virulent strains of *Salmonellae* must change. The impact of non-typhoidal salmonellosis in humans in Nigeria also remains under studied and under reported, especially those caused by *S. Kentucky* ST198. Compounding these concerns is the lack of commercial veterinary or human vaccines against *S. Kentucky* or where vaccines against the broad serogroup C non-typhoidal *Salmonella* (NTS) are available, they are rarely supplied, with no evidence they could be cross-protective. This review emphasizes the emergence and widespread occurrence of MDR *S. Kentucky* strains on the African continent, and discussed risk factors contributing to its spread in Nigeria and the potential public health challenge especially to high-risk immunocompromised individuals.

Keywords: *Salmonella* Kentucky, ST198 strain, multidrug resistant, tuberculosis, HIV/AIDS, Nigeria, Africa

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Salmonella Kentucky est omniprésente dans la plupart des pays africains et les souches multirésistantes (MDR) restent sous-déclarées à travers le continent. Au Nigéria, la pauvreté, les échanges de bétail entre pays, le système d'élevage nomade, l'utilisation aveugle d'antibiotiques et les maladies immunodéprimantes prévalentes telles que le virus de l'immunodéficience humaine/le syndrome d'immunodéficience acquise (VIH/SIDA) et la

tuberculose sont des facteurs qui ont facilité la transmission et complications des infections à *S. Kentucky*. Au cours de la présente décennie, *S. Kentucky* serait le sérotype le plus répandu associé à la volaille au Nigéria, mais très peu de rapports soulignent le risque associé à la consommation de volaille et à l'acquisition de souches MDR *S. Kentucky*. La volaille nigériane est l'un des sous-secteurs les plus commercialisés de l'agriculture nigériane, par conséquent, la présence de *S. Kentucky*, en particulier les souches présentant une résistance antimicrobienne à large spectre, pose un grand risque pour la santé publique. L'absence de suivi, de surveillance, d'isolement et de contrôle adéquats du *S. Kentucky* multirésistant résistera toujours au défi du potentiel d'exportation du sous-secteur de la volaille nigérian et du bétail en général. En tant que nation, les modalités et les actions contre la contrebande de produits de volaille, l'utilisation aveugle d'antibiotiques et le système nomade pour la production de produits laitiers et de boeuf qui favorisent la propagation de souches virulentes de salmonelles doivent changer. L'impact de la salmonellose non typhoïde chez l'homme au Nigeria reste également sous-étudié et sous-signalé, en particulier ceux causés par *S. Kentucky* ST198. À ces inquiétudes s'ajoute le manque de vaccins commerciaux vétérinaires ou humains contre *S. Kentucky* ou là où des vaccins contre les *Salmonella* non typhoïde du séro groupe C sont disponibles, ils sont rarement fournis, sans aucune preuve qu'ils pourraient être de protection croisée. Cette revue met l'accent sur l'émergence et la présence généralisée de souches MDR *S. Kentucky* sur le continent africain, et a discuté des facteurs de risque contribuant à sa propagation au Nigéria et du défi potentiel de santé publique en particulier pour les personnes immunodéprimées à haut risque.

Mots-clés: *Salmonella* Kentucky, souche ST198, multirésistante, tuberculose, VIH/SIDA, Nigéria, Afrique

Introduction:

Salmonella is a Gram negative, non-lactose fermenting and non-sporing facultative anaerobic rod-shaped bacteria belonging to the family Enterobacteriaceae (1). There are over 2,600 different serovars and more than 50 serogroups in the genus *Salmonella* as described in the Kauffman White scheme, and majority of the recognized serovars cause infections in human and animals worldwide (2,3). *Salmonella* can be subdivided into two broad groups with respect to pathogenesis of the bacteria. The first group of serovars is host specific and typically produces systemic disease but in the absence of disease, these strains poorly colonize the intestine of their host and are rarely involved in human food-poisoning (4). Serovars in this group include *Salmonella* Gallinarum and Pullorum in poultry, *Salmonella* Dublin in cattle, and *Salmonella* Typhi and *Salmonella* Paratyphi A, B and C in man (5).

In human, *Salmonella* Typhi and *Salmonella* Paratyphi A are collectively referred to as typhoidal salmonellosis (also called enteric fevers) causing fatal disease of human called typhoid fever and paratyphoid fever respectively (5). The second group of serovars is known as the non-typhoidal *Salmonella* (NTS). These serovars typically produces food poisoning in man and only cause systemic disease under certain conditions, such as during parturition, in infants and young children, elderly, and immunocompromised persons (6). In animals, they are usually asymptomatic, but systemic diseases have been reported in birds in-lay, very young or old animals, or after some viral infections, and in experimental animals challenged with high bacterial colony forming units (4,7).

In 2010, the global incidence of human NTS gastroenteritis was estimated to be 93 million cases, of which 80.3 million were via food-borne transmission, with over 155,000 deaths reported (8,9). In the same year, a separate report on NTS gastroenteritis put the estimate at approximately 3.4 million invasive infections with 681,000 deaths; 57 % of these illnesses and deaths were reported to have occurred in Africa (10). These NTS strains can infect and colonize a broad range of vertebrate animals and most of these lineages are adapted to humans or may be adapted or restricted to particular non-human animal species (5,11).

There are diversities in the epidemiology and host range for NTS serovars and their propensity to cause bloodstream infection and severe human disease varies (6,12). Invasive NTS is associated with several forms of immunocompromised states unlike in typhoidal salmonellosis, for which there are no clear clinical associations with classic immunocompromising conditions (13). These immunocompromising states include; disorders of oxidative cellular killing such as chronic granulomatous disease, in which NTS is described as the commonest cause of bloodstream infection and the third leading cause of infections (14), in children who are homozygous for sickle cell disease (15), and in people with HIV-AIDS, which is worsened in Africa where this disease is driven in part by co-infection with malaria (10).

This review attempts to provide an update information on the epidemiology of *S. Kentucky* in Africa and Nigeria with associated risk factors that may promote the acquisition and propagation of antimicrobial resistant determinants within the serovar in Nigeria and

the impact such widespread strains may have on public health and the Nigerian livestock subsector.

Methodology:

An online search of relevant published materials and bibliographic citations including review articles, original articles, books and conference papers through the period 1990 to 2019 was done on Google, Google scholar, PubMed and African Journals Online (AJOL). The keywords utilized for the search were 'salmonellosis', 'Salmonella Kentucky ST198', 'multidrug resistant S. Kentucky', 'prevalent Salmonella serovar in Nigeria', 'nontyphoidal salmonellosis in Nigeria', 'epidemiology of nontyphoidal salmonellosis in Africa', 'antimicrobial Salmonella pathogen', 'nontyphoidal salmonellosis and HIV infection in Nigeria', 'salmonellosis and tuberculosis infection in Nigeria', 'and Salmonella Kentucky in the Nigerian cattle and poultry subsector'. Reference materials totaling 186 were identified, and following screening of all the materials, 79 reference materials were selected for the review.

Characteristic features of S. Kentucky:

Salmonella enterica subsp. *enterica* serovar Kentucky (antigenic formula 8, 20: i: z6) is a serovar of the O: 8 (C2-C3) serogroup (16,17). *Salmonella* serogroup C consists of highly diverse serovars among which 37 are reported to account for the majority of human *Salmonella* infection as compared to 17 and 11 serovars for serogroups B and D, respectively (6). Currently, four out of the ten most lethal serovars of *Salmonella* in the United States are from serogroup C and strains within this serogroup are multidrug resistant (9). *Salmonella* Kentucky on several occasions has been isolated from healthy poultry, dairy cows and occasionally from people with clinical disease (16,17). *S. Kentucky* was less often identified in human salmonellosis averaging 62 cases per annum in the United States between 1996 to 2004, but rose to 123 cases in 2006; accordingly, the prevalence in chickens rose from 25% in 1997 to approximately 50% in 2007 (18-20).

While the virulence and colonization mechanism of *S. Enteritidis* (serogroup D), and *S. Typhimurium* (serogroup B) have been extensively studied, studies on *S. Kentucky* are few (21). If left unattended to, *S. Kentucky* exhibits the potential to emerge as the most prevalent NTS serovar in human disease as it has acquired virulent chromosomal and plasmid

factors that gives it superior colonization capabilities of its host and environment (22-24). Johnson et al., (22) reported that *S. Kentucky* harbors a *CoIV* virulence plasmid gene which significantly increased its ability to colonize chicken caecum and cause extraintestinal disease through competitive exclusion. Turki et al., (23) identified the presence of virulence *invA/spvC* genes and quorum sensing *sdiA* gene and elucidated the ability of *S. Kentucky* to form biofilms, which is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, and are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced, and which exhibit altered phenotype with respect to growth rate and gene transcription (25,26).

Interestingly, it has been observed that the resistance of biofilm cells to antimicrobials is significantly increased compared with what is normally seen with the same cells being planktonic (27,28). Thus, it is believed that biofilm formation enhances the capacity of pathogenic *Salmonella* bacteria to survive stresses that are commonly encountered both within food processing, as well as during host infection (29). These virulence factors confer on *S. Kentucky* a superior capacity to persist longer in the environment and explains its ability to acquire virulence plasmid genes from avian pathogenic *Escherichia coli* (APEC) and other non-generic bacteria through horizontal gene transfer (22,23).

Salmonella Kentucky ST198 is an emerging risk for foodborne illness across the globe (30). Whole genome sequencing of isolates obtained from five continents (Africa, Europe, Middle East, Asia and North America) showed that multidrug-resistant (MDR) *S. Kentucky* isolates belonged to a single lineage, which was estimated to emerged circa 1989 following the acquisition of the AMR-associated *Salmonella* genomic island (SGI) 1 (variant SGI1-K), which confers resistance to ampicillin, streptomycin, gentamicin, sulfamethoxazole and tetracycline (31). The MDR clone has undergone substitution mutations in the quinolone-resistance-determining regions (QRDRs) of DNA gyrase (*gyrA*) and topoisomerase IV (*parC*) genes, such that most strains carry three QRDR mutations which together confer resistance to ciprofloxacin. Remarkably, each genome of the MDR clone carried a different SGI1-K derivative structure; this variation could be attributed to IS26n mediated insertions and deletions (31). The genome sequence of *S. enterica* serotype Kentucky ST198 isolate 201001922 has been

deposited in GenBank under the accession number CP028357 (31).

Epidemiology of *S. Kentucky* infection in African countries

In time past, the most widely reported serovars associated with invasive non-typhoidal salmonellosis across Africa were *S. Typhimurium* (serogroup B) and *S. Enteritidis* (serogroup D) (14,32). But recent studies are now showing a significant shift in the predominant *Salmonella* serovar associated with poultry and human infections (18, 24, 33). *Salmonella Kentucky* is reported to be the most widely spread serovar on the African continent where virulent strains of this bacteria is reported to have originated, and this is now of global public health concern (6,24).

A multiple-antibiotic resistant strain of *S. Kentucky* with multilocus sequence type (MLST) 198 was reported to have originated from Egypt. Isolates of this strain of *S. Kentucky* manifested high degree of broad range antibiotic resistance especially toward ciprofloxacin which is considered the mainstay drug of choice for *Salmonella* treatment (24,34). Subsequently this clone has been identified in a number of other African countries (24). This strain was originally considered to be travel related but has now been identified in poultry, various food materials and environments. This emerging strain has acquired chromosomal and plasmid factors over time that give it a superior colonization capacity in host animals (22). In Tunisia, 57 strains of *S. Kentucky* were isolated from different sources between 2005 and 2008 and were characterized by their antimicrobial and mercury resistance profiles and other virulent factors (23). A total of 10.6% of these isolates demonstrated multidrug-resistance against 3 to 13 antibiotics with ciprofloxacin resistance occurring in 33% of the human isolates (23).

Salmonella is considered in Morocco as the major cause of food poisoning in the country, although *Salmonella* cases remains underreported. *Salmonella Kentucky* resistant to ciprofloxacin was first identified in an eight-month old child who was admitted at the paediatric department of University Hospital Centre Ibn-Rochd in Casablanca, Morocco, with an acute febrile diarrheal illness (35,36). In a study of the prevalence of drug susceptibility and molecular characteristics of NTS isolated from laying hens in three Moroccan regions; Rabat-Salé-Zemmour-Zaër, Souss-Massa-Drâa and the grand Casablanca, seven *Salmonella enterica* serovars were identified; *S. Enteritidis*

(37.5%), *S. Kentucky* (31.2%), *S. Infantis* (10.9%), *S. Typhimurium* (6.2%), *S. Thompson* (6.2%), *S. Agona* (4.6%), and *S. Amsterdam* (3.1%). Amongst these serovars, *S. Kentucky* showed the highest level of resistance (25%) to the different drugs tested (37).

In a separate study undertaken by Allaoui et al., (38) in 2017 on the prevalence, sensitivity to antibiotics and distribution of *Salmonella* spp. serotypes in broiler turkey in the north-west of Morocco, twenty farms were inspected three times for the purpose of the study. *S. Kentucky* was the most prevalent serovar (33.8%) isolated, followed by *S. Parkroyal* (16.3%) and *S. Agona* (11.3%), and the results of the Salmonellae antimicrobial resistance showed that multi-drug resistant strains (resistant to three or more antibiotics) accounted for 80.6% of the strains isolated; 4.8% were resistant to ceftazidime, ceftriaxone and cefotaxime (38) with *S. Kentucky* and *S. Agona* showing the highest resistance rates to the tested drugs. The risk factors associated with contamination were linked to; the clean-out period, antibiotic treatment, infection of turkey poult at placement, manure storage, keeping sick turkeys in the turkey house, season and age of turkeys at the time of sampling (38). The findings of a widespread antibiotic resistant strains and high prevalence of *S. Kentucky* reported by Allaoui et al., (38), was in accordance with other studies undertaken in other parts of Morocco (37,39-42). These studies established the threat posed by laying hens and turkey to the effective control of human NTS foodborne diseases in Morocco.

In Uganda, Afema et al., (43) reported a high prevalence of *S. Kentucky* in poultry and significantly high isolation rates in humans. Livestock (poultry, ruminant and swine), environmental and human samples were taken from 14 sites in Kampala, the largest city of Uganda. Thirty-two serovars of *Salmonella* were isolated from these sources amongst which were *S. Enteritidis*, *S. Kentucky*, *S. Newport* and *S. Typhimurium*. Interestingly, *S. Enteritidis* was not detected in human wastewater samples and *S. Typhimurium* was poorly isolated from this sample (43). Sensitivity and resistance profile to 15 antimicrobials (amikacin, amoxicillin-clavulanic acid, ampicillin, cefotaxime, cefoxitin, ceftiofur, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole) showed that *S. Kentucky* exhibited extensive multi-drug resistance pattern and had diverse resistance profile from human, environmental and poultry sources (43), for

instance, the *SSuTCipNa* resistance profile was shared by *S. Kentucky* from human, environmental and poultry sources. A deca-resistant profile, *ACKSSuSxTAmCipNa* was unique to poultry, and *S. Kentucky* isolated from poultry were multi-drug resistant, while other serovars found in poultry were pan-susceptible or had low resistance profile (43). Also, shared genotypes and antimicrobial resistance phenotypes were found in NTS from human, livestock and environmental sources, suggesting the occurrence of zoonotic and environmental transmissions (43).

In West Africa, reports from Ghana indicated that the prevalence and antimicrobial resistance pattern for *Salmonellae* was higher in *S. Kentucky*. In two regions of Ghana, fecal, dust, feed and drinking water samples were collected from 75 commercial egg-laying and broiler farms while skin neck samples were collected from broilers representing different flocks at a local slaughterhouse. Of the 200 samples collected, *Salmonella* was detected in 94 of them representing 47% with an overall flock prevalence of 44.0%. Sixteen serovars of *Salmonella* were identified with *S. Kentucky* the most prevalent (18.1%) followed by *S. Nima* (12.8%), *S. Muenster* (10.6%), *S. Enteritidis* (10.6%) and *S. Virchow* (9.6 %). All the strains of *S. Kentucky* were reported to show multidrug resistance (two or more antimicrobials) including ciprofloxacin, nalidixic acid and tetracycline (44).

In Senegal, recent reports on the prevalence and presence of *S. Kentucky* are sparse but earlier findings showed fluctuations in their isolation rates. A total of 1623 clinical isolates of *Salmonella* belonging to 229 serotypes were received by the Senegalese Reference Center for Enterobacteria from January 1999 to December 2009 (45). The most common serotypes isolated were *S. Enteritidis* (19%), *S. Typhi* (8%), *S. Typhimurium* (7%) and *S. Kentucky* (4%). There were noticeable significant increases in the prevalence of resistance to amoxicillin (0.9% in 1999 to 11.1% in 2009) and nalidixic acid (0.9% in 1999 to 26.7% in 2009) in NTS serotypes (45). The constant threats that arise from sudden increase in emergence and prevalence of new serovars of *Salmonellae* are often due to unknown or unclear reasons (46).

Epidemiology of *S. Kentucky* infection in Nigeria and the concern

Nigeria is reported to have widespread presence of *S. Kentucky* serovar, and the multidrug resistant strain ST198 that is now of

global concern has been isolated (24,33,47). Useh et al., in 2016 (48) reported the findings of a study undertaken in southwest and north central Nigeria where epidemiological samples were collected, with *S. Kentucky* the most common serotype isolated from poultry in these regions, followed by *S. Zega* and *S. Hertston*, which have previously been isolated from diarrheal cases among children in Niger State. *Salmonella* Enteritidis, which was thought to be the most common serotype associated with poultry worldwide, was absent in the study.

Another extensive and elaborate study cutting across all the six geopolitical zones in Nigeria was undertaken (49) with representative samples of five different types (litter, dust, faeces, feed and water) collected from commercial chicken layer farms in selected States in these regions. In this survey, *S. Kentucky* was the most widely spread serovar with prevalence of 16.2% and highest isolation from faeces (23%) and feeds (22.7%), followed by *S. Poona* (5.6%) and *S. Elisabethville*. Also, *S. Kentucky* was distributed across the country, whereas the other less frequent serovars had a more circumscribed diffusion (49). The usual high presence in faeces also indicated high environmental contamination that allowed the pathogen to persist within the poultry flock.

In a separate study involving three southwestern States (Lagos, Ogun and Oyo), Mshelbwala et al., in 2017 (50) reported that *S. Kentucky* was the second most prevalent serovar with prevalence rate of 24.3%. The study reported further that although this serovar was less often associated with mortality in birds, the finding seems to contradict this norm (50) as sampled and challenged birds showed classical lesions consistent with that exhibited by the host specific serotypes. The findings of Fagbamila et al., (49) and Mshelbwala et al., (50) are amongst the most recent reports regarding *S. Kentucky* in the Nigerian poultry subsector. The risk factors reported in these studies that allowed for widespread presence of this serovar included poor biosecurity, poor sanitation measures, and lack of adequate monitoring and surveillance in the farms.

Earlier reports by Raufu et al., (47, 51) identified the presence of the highly pathogenic *S. Kentucky* ST198 strain that encode genes resistant to multiple antimicrobials. In the study, *S. Kentucky* isolates from chicken and farm environmental sources (litter, faeces, water) in Ibadan (southwestern) and Maiduguri (northeastern) Nigeria were screened to estab-

lish for the presence of ciprofloxacin resistant genes (*S. Kentucky CIPR* strain) using pulse field gel electrophoresis (PFGE) and minimum inhibitory concentration (MIC) in 2007, 2010 and 2011. Six percent of the samples were positive for *S. Kentucky* and all resistant to nalidixic acid and ciprofloxacin (47). The isolates showed genotypic relatedness to *S. Kentucky CIPR* strain based on *XbaI* PFGE fingerprinting, indicating the presence of wide-spread *S. Kentucky CIPR* clone within poultry and farm environment in these regions (47).

Nigeria is the most populous and one of the largest countries on the African continent, with a total geographical area of 923,768 square kilometers (52). The estimated population of the country in 2013 was 174.5 million people with a population growth rate of 3% per annum and the National Population Commission (NPC) put the estimate at over 193 million in 2016 (53,54). The Nigerian economic statistics reveal an economic growth rate that averaged over 7% per annum in recent decades, making Nigeria one of the fastest growing economies on the continent (55). Nonetheless, this growth has neither reduced poverty nor created a stable environment for jobs thus; unemployment is still very high, and more than 60% of the population lives below the poverty line (56).

Poultry and cattle production play significant roles in the epidemiology of *Salmonellae* in Nigeria, because they act as reservoirs, and excrete the organism in their faeces, and their products are easily contaminated by infected animals (49,57,58). The Nigerian poultry industry has expanded rapidly in recent years despite encountering several shortcomings like the global financial crisis, inadequate credit facilities, a weak level of biosecurity and a relatively low level of production (59). The Nigerian poultry industry rose from approximately 150,700 million chickens in 2005 to 192,313 million in 2010 (60), thus it is one of the most commercialized and growing subsectors of Nigerian agriculture (53,61); and also accounts for about 25% of the country local meat production.

The advantages of poultry production over other livestock production can be explained by the fact that poultry birds are good converters of feed into useable protein in meat and eggs, production costs per unit remain relatively low, and return on investment is high. Therefore, subsistence farmers need a relatively small amount of capital to start a poultry farm. Also, poultry meat is tender and acceptable to consumers, regardless of their religious backgrounds.

Furthermore, the production cycle is quite short, so capital is not tied up for too long, and eggs, which is a major products of poultry farming, are more affordable for the common people than other sources of animal protein (62,63). This makes the poultry subsector an essential part of the Nigerian economy and a good source of high-quality protein for the teeming population.

In food-producing animals, especially poultry, infection with *Salmonella* has a direct impact on the global marketing of the respective livestock animals and livestock-derived food products (64). The high rate of production of poultry in Nigeria signifies a high rate consumption of its products; however, this also means a concomitant increase in the cases of *Salmonella*-associated gastroenteritis (65) and thus makes *S. Kentucky* ST198 an imminent threat to the poultry subsector and a limiting factor to its export potential. Thus, prevention of *Salmonella* infections is important for the profitable expansion of the poultry industry. Despite this assertion, an effective prevention and control measure cannot be undertaken unless the status of the disease and epidemiology are well elucidated (62). There are very few African countries that report surveillance data on *Salmonella* and as such, very limited information is available on *Salmonella* isolation for the Africa continent (58,66).

One of the factors that may contribute to the proliferation of bacteria strains resistant to multiple antimicrobials in Nigeria would be the indiscriminate use of antimicrobials in the poultry subsector. Indiscriminate use of antimicrobials in any environment creates selection pressures that favours the survival of antibiotic-resistant strains (67) and in Nigeria, the practice of routinely giving antimicrobials to domestic livestock for growth promotion and prophylaxis is an important factor in the emergence of antibiotic-resistant bacteria in the food chain (65,68). The occurrence of ciprofloxacin resistant serovar is of economic importance since fluoroquinolone resistance has implications for both veterinary and human therapy ciprofloxacin is a mainstay drug in the treatment of salmonellosis thus; the abuse of these medications in poultry could result in the emergence of resistant zoonotic bacteria (62).

Risk factors for *S. Kentucky* infection

Nomadic system of cattle rearing

Food animals such as cattle play significant roles in the epidemiology of *Salmonella*, because they act as reservoirs and

excrete *Salmonellae* in their faeces (57), therefore, meat and meat products that are contaminated during slaughter and processing, pose severe health risks, and infections can also result from cattle contact (69,70). Beef from cattle is considered an important source of salmonellae in Nigeria as they are widely consumed, and reports have shown that they are source for transmission of zoonotic *Salmonella* serovar including *S. Kentucky* to man. The potential for zoonotic transmission is further increased given limited capacity for sanitation, nomadic system of cattle production and co-mingling of this livestock with human population (71).

In 2017, Fashae et al., (71) compared phenotypic and genotypic characteristics of *Salmonellae* isolated from cattle and humans in a study of faecal samples from diarrhoeic patients, healthy population, cattle beef at slaughter, farms, and markets. The *Salmonella* isolates were serotyped and their antimicrobial susceptibility pattern determined (71) while whole genome sequencing (WGS) of selected isolates and bioinformatics analysis were used to identify the multilocus sequence type (MLST), plasmid replicons, antimicrobial resistance genes, and genetic relatedness by single nucleotide polymorphism (SNP).

The MLST sequence types (ST) include ST 584, ST 198, ST 562 and ST 512 for *S. Colindale*, *S. Kentucky*, *S. Rubislaw* and *S. Urbana* respectively. The findings in this study showed multiple antibiotic resistant serovar exhibited by *S. Kentucky* and *S. Typhimurium*. In the cattle market, both *S. Kentucky* and *S. Ealing* co-predominated (40% each) (71). There was an overlapping of *Salmonella* serovars between humans and cattle and also between the diarrhoeic patients and healthy women, and such serovars included *S. Colindale*, *S. Give*, *S. Corvallis*, *S. Oranienburg* and *S. Kentucky*. Majority (83.3%) of the resistant isolates obtained from the healthy women comprised six serovars including *S. Kentucky*, while all the three resistant isolates from cattle were *S. Kentucky* (71), and all the ciprofloxacin resistant isolates belonged to *S. Kentucky*. Furthermore, majority (57.1%) of the multidrug resistant (resistance to ≥ 3 antimicrobials) isolates were *S. Kentucky* (71). The findings of this study buttressed the fact that in Nigeria, cattle are important reservoir of diverse serovars of *Salmonellae* including the highly virulent multiple antimicrobial resistant *S. Kentucky* strains.

Inter country livestock trade

Trading of livestock between African

countries is an important means for the transmission of zoonotic pathogens between nations. Despite the huge potentials of the Nigerian poultry subsector, the 330 billion Naira chicken meat segment of the industry, is still struggling to grow because of the smuggling of large volumes of imported chicken products into her markets (72). The poultry industry estimates that about 1.2 million metric tons of poultry products are smuggled into Nigeria annually, primarily from Brazil and Asia, and although the government of Nigeria has placed a ban on the importation of poultry products, implementation has not been effective (72).

The Western and Central African countries are reported to import large quantities of frozen poultry products from the European Union (EU). Between 1996 and 2003, chicken imports from the EU to ECOWAS countries increased from 125,000 to 860,000 metric tons. Benin was the major importer, followed by Ghana, Nigeria, Senegal, Togo, and Côte d'Ivoire. Yet about 90 percent of Benin's poultry imports are informally re-exported to Nigeria as reported by the World Bank. The illegal importation of these poultry products, porosity and poor manning of the Nigeria border and inadequate surveillance policy on *Salmonella* spp. play important role in the acquisition and transmission of the *S. Kentucky* serovar to the country.

Indiscriminate use of antimicrobials

The use antimicrobials cannot be overlooked by their proven importance for a sustainable livestock production especially in the control and treatment of livestock infections and their use as growth promoters (73). The use of antimicrobials in any environment creates selection pressures that favors the survival of antibiotic-resistant strains (67), and in Nigeria, the practice of routinely administering antimicrobials in the livestock industry as growth promoters and prophylaxis is an important factor that promotes the emergence of antibiotic-resistant bacteria in the food chain with concomitant socioeconomic and public health risk (65,68,73). Furthermore, the abusive use of antimicrobials in many parts of the world is recognized as key drivers for the emergence and spread of antimicrobial resistance (74-77).

In a study conducted by multi-stage sampling technique of 454 farmers in 11 communities within 11 Local Government Areas (LGAs) of Kaduna and Oyo States in northern and southern Nigeria respectively to ascertain antimicrobials usage and practices by livestock

farmers in these regions of the country (73). Antimicrobial usages were collected through interviews, questionnaire and focus group discussions. The study revealed that antimicrobials were widely distributed, easily accessible and commonly used in livestock production, and antimicrobials that are crucial in human therapy including flouroquinolones were widely used in animals as prophylactics. Also, potentially harmful antimicrobials including furazolidones and chloramphenicol which have been banned for use in humans and animals were freely marketed and used in livestock production (73). Most of the respondents in the study acknowledged that it was standard practice for drugs and antimicrobials to be prescribed and administered to animals, but on the farm, they buy and administer antimicrobials without professional consultation (73).

In another study, Ogunleye et al., (78) in 2010 reported the widespread occurrence of multidrug resistant *Salmonella* serovars from poultry disease outbreaks in two southwestern Nigerian states (Oyo and Ogun) where a total of 73.3% of *Salmonella* isolates tested were multi-drug resistant (resistance to four or eight antibiotics) which included resistance to ciprofloxacin, nalidixic acid, chloramphenicol, streptomycin, kanamycin, ampicillin, neomycin, and tetracycline, and specifically, 63.4% were ciprofloxacin resistant (78). The practice of avoiding veterinary personnel and laboratory investigations in the diagnosis of disease prior to institution of antimicrobial therapy could lead to improper usage that promotes the development of antimicrobial resistance in bacterial strains (73).

Use of antimicrobial residue in animal feed

The indiscriminate use of therapeutic and prophylactic antimicrobials administered in feedstuffs in the Nigerian livestock industry is a managerial practice that gives opportunity for antimicrobials to enter the human food chain (through eggs, milk, meat, etc.) especially when drug withdrawal periods are not adhered to (79). Medicated feedstuffs are primarily a mixture of animal feed and veterinary medicinal products, which are usually authorized for the animal species for which the feed is intended and contains high antimicrobial concentrations depending on its aim, physiological status and state of health of the animal.

The use of these feedstuffs accompanied by the tendencies for human error in compounding and contamination of the antibiotic negative feed with antimicrobials, improper production, poor handling and storage

practices, little proportions of medicated feedstuff being left in production line or storage tanks leading to the cross-contamination of next batches of antimicrobial negative feed, pose severe risks and safety concerns to both animals and man (79), and promote the spread of antibiotic resistant pathogens such as *S. Kentucky* ST198.

Potential challenges of *S. Kentucky* NTS to HIV/AIDS patients

Data from the United Nations Programme on human immunodeficiency virus and the acquired immune deficiency syndrome (UNAIDS) put the estimate number of people in Nigeria living with HIV in 2018 at 1.9 million (80) which makes the nation the second largest HIV epidemic in the world and approximately 160,000 people died from AIDS related illnesses in Nigeria in 2017 (81). Although new survey indicates that Nigeria has HIV prevalence among adults (aged 15 - 49 years) at 1.5%, this was previously estimated at 2.8% (82), and the recent drops in prevalence estimates for Nigeria were attributed to better surveillance (83). Six States in Nigeria that account for 41% of people living with HIV are Kaduna, Akwa Ibom, Benue, Lagos, Oyo, and Kano (84) but the prevalence of HIV is highest in Nigeria southern states (known as the south-south zone) at 5.5% and lowest in the southeast zone with a prevalence of 1.8%. Previous reports showed that there are higher rates of HIV in rural areas (4%) than in urban ones (3%) (85).

Reports have indicated that the use of antimicrobials for growth-promotion, prophylaxis, and treatment of food animals increases the prevalence of resistance in human pathogens, particularly NTS in people living with HIV/AIDS. Researches to ascertain the prevalence of NTS amongst people living with HIV/AIDS has been undertaken in the country. In Ekiti State, a study was undertaken to evaluate the relationship between consumption of poultry products and prevalence of HIV/AIDS, with collection of 100 stool and 50 blood samples from a total of 150 HIV/AIDS patients attending Federal Medical Centre, Ido-Ekiti, and 50 faecal samples from broiler chickens for culture and identification of NTS as well as their antimicrobial susceptibilities and similarities (86). Eighteen NTS isolates were identified from the human with a prevalence of 12% while four NTS isolates were identified from poultry broiler-chicken with prevalence of 8%. Isolates from broiler chickens had a higher average resistance to

seven commonly prescribed antibiotics including ciprofloxacin (78.6%) when compared to the human isolate (53.9%). Furthermore, most of the isolates from human and broiler chicken showed resistance to more than one antibiotic expressing the concern that antimicrobial resistance is wide spread in both poultry and persons living with HIV/AIDS (86).

Potential challenges of *S. Kentucky* NTS to tuberculosis patients

The lack of epidemiological data on the impact of NTS on tuberculosis (TB) or records implicating opportunistic infections caused by antimicrobial resistant *Salmonellae* on TB patients is rare. In 1995, a 33 years old woman with recurrent *Salmonella* Enteritis infection was found to have underlying hepatic tuberculosis (87). Ciprofloxacin, ceftriaxone, penicillin and chloramphenicol were among the antimicrobials administered that failed to eradicate the recurrent nontyphoidal salmonellosis. Over the past decades, *S. Kentucky* has been identified as most prevalent multi-drug resistant *Salmonella* serovar in human salmonellosis infection (22, 24).

The World Health organization prioritizes fluoroquinolone resistant *Salmonellae* amongst the high-risk pathogens to human health (31) and because mycobacterium, causative agent for TB, are fairly susceptible to quinolones, repeated ciprofloxacin therapy which is the main stay drug for treatment of Salmonellosis can mask early and proper diagnosis of TB (87). Since antimicrobial resistance, HIV and poverty are the backbone for the spread of tuberculosis globally (88), and with these conditions endemic in Africa and Nigeria, it becomes pertinent for the Nigerian government and health organizations to draft up policies as they design national TB control programme that will prevent possible complications caused by this pathogen.

It should be noted that Nigeria is ranked among the 30 high TB burden countries of the world and one of the top three of ten countries that accounted for 80% of the total gap between TB incidence and reported cases in 2017 (89,90). Studies from settings outside Nigeria reveal poor governance practices as responsible for the increased scourge in the country. The lack of specific TB legislation, inconsistencies in the enforcement of policies on isolation of TB patients in health facilities (91,92), Low government funding (93,94), inadequate trained personnel and lack of public awareness (95), poor integration into the general health system, weak programme impl-

mentation, sub-optimal quality of care in the private sector, and insufficient advocacy around TB (88) limited the strategic vision and responsiveness of TB control programmes. Multidrug resistant (MDR) TB cases have made this menace terrifying for patients. Taking cognizance of the high incidence and epidemiologic significance of occurrence of MDR-TB strains, it is imperative that determination of MDR-TB burden and complications that may be due to *S. Kentucky* ST198 in acquisition of MDR-TB strains in patients be elucidated to inform proper case management and overall planning of TB control activities.

Conclusion:

The World health organization prioritizes fluoroquinolone resistant *Salmonellae* amongst the high-risk pathogens to human health and *S. Kentucky* ST198 wears this cap. The genomic/genetic efficiency of this strain has put it in the frontline as an emerging threat to public health and the commercial livestock subsector. The lack of epidemiological data, proper monitoring and surveillance systems, and indiscriminate use of antimicrobials in Nigeria and the African continent has enabled the widespread propagation of this serovar. On a continent where poverty and immuno-compromising diseases such as HIV/AIDS and tuberculosis are endemic, *S. Kentucky* ST198 has shown explicit potential as an ideal opportunistic non-typhoidal *Salmonella* serovar that could compound these conditions.

The private sectors, government agencies and international organizations on the African continent are key players that can be involved in forestalling the propagation of this strain in the environment. Strategic policies can be made and informed decisions taken to curtail the spread of *S. Kentucky* ST198, for example, there should be regulations and restrictions on the use and over-the-counter sale of veterinary antimicrobials, provision of biosecurity facilities and trained personnel at inter country livestock markets and meat slaughtering/processing plants, adequate surveillance, monitoring and data recording practices are measures that can limit its spread.

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**Original Article****Open Access****Co-infections of MERS-CoV with other respiratory viruses in Saudi Arabia***¹Al-Quthami, K., ²Al-Waneen, W. S., and ³Al Johnyi, B. O.¹Regional Laboratory, Makkah, Saudi Arabia²National Centre of Agricultural Technology, King Abdulaziz City for Science and Technology, Saudi Arabia³King Abdulaziz University, Saudi Arabia*Correspondence to: khmrqu@hotmail.com**Abstract:**

Background: The Middle East Respiratory Syndrome (MERS) is a viral respiratory disease caused by a member of the coronaviruses called Middle East Respiratory Syndrome Coronavirus (MERS-CoV). The co-infections of MERS-CoV with other respiratory viruses have been documented in rare cases in the scientific literature. This study was carried out to determine whether co-infection of MERS-CoV occurs with other respiratory viruses in Saudi Arabia.

Methods: Nasopharyngeal swabs samples of 57 MERS-CoV positive outpatients were collected using flocked swabs. Nucleic acid was extracted from each sample using commercial NucliSens easyMAG system. Amplification was performed by multiplex RT-PCR using Fast Track Diagnostics Respiratory Pathogen 33. Data were analyzed with SPSS software version 19 and comparison of variables was done with Fisher Exact test, with p value <0.05 considered significant.

Results: Six of the total 57 MERS-CoV patients (35 males, 22 females) were positive for co-infection of MERS CoV with other respiratory viruses, giving a prevalence rate of 10.5%, with 14.5% (5/35) in males and 4.5% (1/22) in females (OR=3.500, 95% CI=0.3806-32.188, $p=0.3889$). The prevalence of co-infections was significantly higher among non-Saudis (23.8%, 5/21) than Saudis (2.8%, 1/36) (OR=0.09143, 95% CI=0.009855-0.8485, $p=0.0217$), and among the age group 18-34 years (25%, 3/12) than other age groups ($X^2=3.649$, $p=0.1613$). Human rhinovirus (HRV) was found in 2 of the 6 (33.3%) patients with co-infection while the other viruses were found in each of the remaining 4 patients.

Conclusion: Our study confirms that MERS-CoV co-infects with other respiratory viruses in Saudi Arabia.

Keywords: MERS-CoV; URTI; Co-infection; Coronavirus

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Co-infections de MERS-CoV avec d'autres virus respiratoires en Arabie saoudite*¹Al-Quthami, K., ²Al-Waneen, W. S., et ³Al Johnyi, B. O.¹Laboratoire régional, La Mecque, Arabie saoudite²Centre national de technologie agricole, ville du roi Abdulaziz pour la science et la technologie, Arabie saoudite³King Abdulaziz University, Arabie saoudite*Correspondance à: khmrqu@hotmail.com**Abstrait:**

Contexte: Le syndrome respiratoire du Moyen-Orient (MERS) est une maladie respiratoire virale causée par un membre des coronavirus appelé coronavirus du syndrome respiratoire du Moyen-Orient (MERS-CoV). Les co-infections de MERS-CoV avec d'autres virus respiratoires ont été documentées dans de rares cas dans la littérature scientifique. Cette étude a été réalisée pour déterminer si la co-infection du MERS-CoV se produit avec d'autres virus respiratoires en Arabie saoudite.

Méthodes: Des écouvillons nasopharyngés de 57 patients ambulatoires positifs au MERS-CoV ont été prélevés à

l'aide d'écouvillons floqués. L'acide nucléique a été extrait de chaque échantillon en utilisant le système NucliSens easyMAG commercial. L'amplification a été réalisée par RT-PCR multiplex en utilisant Fast Track Diagnostics Respiratory Pathogen 33. Les données ont été analysées avec le logiciel SPSS version 19 et la comparaison des variables a été effectuée avec le test Fisher Exact, avec une valeur $p < 0,05$ considérée comme significative.

Résultats: Six des 57 patients MERS-COV (35 hommes, 22 femmes) étaient positifs pour la co-infection de MERS CoV avec d'autres virus respiratoires, donnant un taux de prévalence de 10,5%, avec 14,5% (5/35) chez les hommes et 4,5% (1/22) chez les femmes (OR 3.500, 95% CI 0.3806-32.188, $p=0.3889$). La prévalence des co-infections était plus élevée chez les non-saoudiens (23.8%, 5/21) que chez les saoudiens (2.8%, 1/36) (OR=0.09143, 95% CI=0.009855-0.8485, $p=0.0217$) et parmi le groupe d'âge de 18 à 34 ans (25%, 3/12) que dans les autres groupes d'âge ($\chi^2=3.649$, $p=0.1613$). Le rhinovirus humain (VRC) a été trouvé chez 2 des 6 (33,3%) patients co-infectés tandis que les autres virus ont été trouvés chez chacun des 4 patients restants.

Conclusion: Notre étude confirme que le MERS-CoV co-infecte avec d'autres virus respiratoires en Arabie Saoudite.

Mots-clés: MERS-CoV; URTI; Co-infection; Coronavirus

Introduction:

The Middle East Respiratory Syndrome is a viral respiratory disease caused by member of a coronavirus called Middle East Respiratory Syndrome Coronavirus (MERS-CoV). The virus was first discovered in Saudi Arabia in September 2012. The health officials carried out a retrospective investigation later and identified that the first known cases of MERS occurred in Jordan in April 2012 (1). After its first identification in Saudi Arabia, MERS rapidly spread to other countries, resulting in health crises at the international level. As of June 2015, MERS-CoV had spread to more than 20 countries (2,3). The morbidity and mortality caused by MERS-CoV were particularly alarming, with mortality rate approaching 60% (4). By June 19, 2015, a total of 1035 confirmed cases of MERS-CoV were reported by the Ministry of Health in Saudi Arabia with 458 deaths (case fatality rate of 44%).

The clinical spectrum of MERS-CoV disease ranges from asymptomatic or mild respiratory symptoms to severe acute respiratory disease and ultimately death. The typical symptoms of MERS include cough, shortness of breath and fever (5). Pneumonia is a common finding but is not always present. Others such as gastrointestinal symptoms like diarrhea have also been reported. About three to four out of every ten patients or rather 36% of patients reported with MERS have succumbed to the illness (6). In its severe form, MERS can cause respiratory failure that will warrant mechanical ventilation support in an intensive care unit. Severe disease occurs in older populations, immunosuppressed, and those with underlying chronic diseases such as diabetes, chronic lung disease and cancer (2).

The MERS-CoV virus is a zoonotic type transmitted from animals to humans. The studies on the origins of the virus are still inconclusive, but according to an analysis of the different genomes of the virus, it is believed that the virus originated in bats and was later

transmitted to camels in the distant past (7). The zoonotic transmission is still not fully understood, however, studies on the disease point towards camels as the major reservoir host for the virus and thus the source of infection in humans. Various strains of the virus, which are identical to the human strains, have been isolated from camels in most countries, such as Oman, Qatar, Egypt, and Saudi Arabia.

There is yet no vaccine or specific treatment available at the moment for the disease. Treatment is basically supportive and entirely dependent on the patient's clinical condition at the time of presentation. Standard precautions should be observed by people visiting farms, barns or other places where contact with camels is highly probable. These precautions include regular hand washing before and after touching animals and importantly, avoiding contacts with sick animals (8). Consumption of raw or undercooked meat and milk is a risk factor for zoonotic transmission, therefore, animal products should be processed appropriately through cooking or pasteurization and handled with care to avoid cross-contamination with uncooked food items. Although, camel products have proven to be nutritious, they should only be consumed after cooking or heat treatment or pasteurization (9). Persons who are immunocompromised or have renal failure, diabetes and chronic lung disease are considered to be at high-risk for severe MERS-CoV infection. These groups of individuals should hence avoid contact with camels or consumption of raw camel products (2).

Co-infections of MERS-CoV with other respiratory viruses have been documented in rare cases in the scientific literature (11,12), however, co-infection of other respiratory viruses within themselves is common and has been well documented (13). Also, some studies have reported that co-infection of different variants of MERS-CoV does occur (14), but there is very little data available to date to confirm co-infections of MERS-CoV with other respiratory viruses. This study was therefore conducted to investigate whether coinfection of MERS-CoV

virus occurs with other respiratory viruses, the objective of which is to determine the prevalence rate of co-infections of MERS-CoV with other respiratory viruses in Saudi Arabia.

Materials and method:

Study setting and design

The study was a descriptive cross-sectional design conducted in Microbiology Department, King Abdulaziz University, Jeddah, Saudi Arabia, from the period of December 2015 to May 2016.

Subjects and sample collection

A total of 57 subjects (35 males and 22 females) of all age groups positive for MERS COV infection in the outpatient department of five regional laboratories (that perform diagnostic tests only), distributed in different regions of the Kingdom of Saudi Arabia, were enrolled for the study. Ethical approval was obtained from the Scientific Research Ethics Committee of the King Abdulaziz University, Saudi Arabia. Informed consent of each adult subject and assent of children were also obtained.

Nasopharyngeal swab was collected from each subject with flocked swab, which was inserted into the nostril towards the pharynx until resistance was felt. Swab was then rotated three times to obtain samples containing epithelial cells. All swabs were put in virus transport media (VTM) and transported at -80°C to the main Scientific Research Laboratory of King Abdulaziz University, Saudi Arabia, for processing.

Data collection

All the clinical and laboratory data of subjects were collected via the Health Electronic Surveillance Network (HESN), the national surveillance network for reporting infectious diseases and outbreaks in Saudi Arabia.

Nucleic acid extraction

Nucleic acid was extracted from 200 µl of the nasopharyngeal sample using NucliSens easyMAG system (BioMérieux, USA) following the manufacturer's instructions. The extracted nucleic acid was either amplified immediately or stored at -80°C until needed.

Multiplex RT-PCR with Fast Track Diagnostics Respiratory Pathogen 33

The extracted nucleic acid was amplified by a one-step multiplex real time PCR using the FTD Respiratory Pathogens (FTD-RP33) assay version 3 which consists of eight-tube multiplex for the detection of respiratory pathogens genes (viruses and bacteria) by TaqMan® technology.

Tube 1 multiplex assay was for influenza A (FluA), influenza B (FluB), influenza A (H1N1) swine lineage, and rhinovirus; tube 2 assay was for coronavirus NL63, coronavirus 229E, coronavirus OC43, and coronavirus HKU1; tube 3 assay was for parainfluenza 2, parainfluenza 3, parainfluenza 4, and internal control; tube 4 assay was for parainfluenza 1, human metapneumovirus A/B, bocavirus, and *Mycoplasma pneumoniae*; tube 5 assay was for respiratory syncytial viruses A/B, adenovirus, enterovirus, and parechovirus; tube 6 assay was for *Chlamydia pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type B, and *Staphylococcus aureus*; tube 7 assay was for *Klebsiella pneumoniae*, *Legionella pneumophila*/*Legionella longbeachae*, *Salmonella* spp, and *Pneumocystis jirovecii*; while tube 8 assay was for *Moraxella catarrhalis*, *Bordetella* spp (except *Bordetella parapertussis*), *Haemophilus influenzae*, and influenza C (FluC).

Five microliters of the template were used for each of the eight multiplex PCR assays. An internal control (BMV), which was included in the FTD kit, was run along each patient sample in the PCR assay. The thermocycling conditions were as follows; 95°C for 10 min and 50°C for 15 min, followed by 40 cycles of 95°C for 8 s and 60°C for 34 s.

Statistical analysis

Data were analyzed using SPSS software version 19 (SPSS, Chicago, IL, USA) and the openly available statistical software environment R version 2.10.1. Comparison of data was done using Fisher Exact test, and p value < 0.05 was considered significant.

Results:

Epidemiological statistics

Of the 57 patients tested (35 males, 22 females), 6 were confirmed positive for one or more respiratory viruses, given a co-infection prevalence rate of 10.5%. The prevalence rate of 14.5% (5/35) in the males was higher (though not significantly) than the rate of 4.5% (1/22) in the females (OR=3.500, 95%CI=0.3806-32.188, $p=0.3889$). The co-infection rate of 23.8% (5/21) among non-Saudis was significantly higher than 2.8% rate (1/36) among Saudis (OR=0.09143, 95%CI=0.009855-0.8485, $p=0.0217$). Co-infection of MERS-CoV with other respiratory viruses progressively decreased with age, with prevalence higher in patients below 50 years (14.7%, 5/34) than patients above 50 years (4.3%, 1/23) (OR=3.79 95% CI=0.4928-34.853, $p=0.3846$), with rate being 25% (3/12) in people aged 18-34 years

Table 1: Demographic characteristics of MERS-COV patients with co-infection of respiratory viruses

Demographic characteristics	No of patients with MERS-CoV (n=57)	No of patients with co-infection (%) (n=6)	X ²	OR	95% CI	p value
Gender						
Male	35	5 (14.3)	-	3.500	0.3806 -32.188	0.3889
Female	22	1 (4.5)				
Age group (years)						
18 - 34	12	3 (25.0)	3.649	-	-	0.1613
35 - 49	22	2 (9.1)				
≥ 50	23	1 (4.3)				
Age group (years)						
< 50	34	5 (14.7)	-	3.793	0.4928 - 34.803	0.3846
≥ 50	23	1 (4.3)				
Nationality						
Saudis	36	1 (2.8)	-	0.09143	0.009855 - 0.8482	0.0217*
Non-Saudis	21	5 (23.8)				

X²=Chi square; OR=Odds Ratio; CI=Confidence Interval; MERS-CoV=Middle East Respiratory Syndrome-Coronavirus; *=statistically significant

Table 2: Respiratory viruses identified in patients positive for MERS-COV

Patient code	Virus detected
3015201185	HBoV
3015201197	Cor43
3015212494	HRV, Cor229, FluA, H1N1
3015215102	HRV
3015215107	RSV
3015215113	HMPV

Cor43 =Coronavirus OC43; Cor229 = Coronavirus 229E; FluA = Influenza A; H1N1 = Influenza A swine lineage; RSV = Respiratory Syncytial Virus A/B; HMPV = Human Metapneumovirus A/B; HRV = Human rhinovirus; HBoV = Human bocavirus.

Table 3: Demographic statistics of patients co-infected with other viruses indicating each virus

Viruses	Number of patients	Age group (years) (number and %)			Gender (number and %)	
		18-34	35-49	over 50	Male	Female
Total number						
RSV	1	0	0	1 (100)	0	1 (100)
HRV	2	1 (50)	1 (50)	0	1 (100)	0
HBoV	1	1 (100)	0	0	1 (100)	0
HMPV	1	1 (100)	0	0	1	0
Cor229	1	1 (100)	0	0	1 (100)	0
Cor43	1	0	1 (100)	0	1 (100)	0
FluA	1	1 (100)	0	0	1 (100)	0
H1N1	1	1 (100)	0	0	1 (100)	0

Cor43 =Coronavirus OC43; Cor229 = Coronavirus 229E; FluA = Influenza A; H1N1 = Influenza A swine lineage; RSV = Respiratory Syncytial Virus A/B; HMPV = Human Metapneumovirus A/B; HRV = Human rhinovirus; HBoV = Human bocavirus.

followed by those patients aged between 35-49 years (9.1%, 2/22) and patients above 50 years (4.3%, 1/23) (X²=3.649, p=0.1613) (Table 1). Two of the six patients with co-infection were admitted on suspicion of MERS CoV, based on symptoms while and two were due to contact

with the camel. Of those admitted, two died, another one was isolated while the two patients admitted due to contact with camel recovered and were discharged. Of the 6 patients, 4 are from Riyadh city, and 1 each from Jeddah and Kharj.

Viral prevalence and clinical profiles of patients

RT-PCR was utilized in the initial test for MERS-CoV in the entire sample collected. Consistent with the existing circulation pattern and prevalence of MER-CoV in Saudi, 6 samples tested positive for co-infection with other respiratory viruses indicating few nasal carriage of viruses. As illustrated in Table 2, 5 patients were co-infected with a single respiratory virus while 1 patient was co-infected with four respiratory viruses. Human rhinovirus (HRV) was the most prevalent, infecting two of the six patients, while the other viruses infected single patient. In the patient with MERS-CoV, there was co-infection with HRV, Cor229, FluA, and H1N1 (Table 2). The only virus found in a female patient was RSV while the rest were found in the males. The co-infection was more frequent in age group 18-34 years followed by 35-49 years (Table 3).

Discussion:

In the current study of 57 MERS-CoV positive outpatients in these regional laboratories in Saudi Arabia, co-infection prevalence rate of MERS-CoV with other respiratory viruses was 10.5%. This finding is consistent with those of previous studies indicating that co-infection of MERS-CoV with other respiratory viruses has been documented to be low (11, 12). Also, some studies have reported that co-infection of different variants of MERS-CoV do occur (14), however no virus co-infection was detected in individuals below 24 years, showing that prevalence of co-infection of MER-CoV with other viruses is rare in children.

In this study, the frequency of co-infection was higher (though not statistically significant) in the males (14.5%) than the females (4.5%). The higher prevalence in the male could be explained by the lifestyle risk factors such as smoking which could expose them to upper respiratory tract infections. Co-infection was significantly higher ($p=0.0217$) among the non-Saudis (23.8%) than the Saudis (2.8%), and our data showed that most of the co-infections were among immigrants from Yemen. Most of the cases (4/6, 66.7%) of co-infections were observed in patients from Riyadh city while one co-infected patient each was from Jeddah and Kharj. This occurrence of MERS-CoV co-infection in Riyadh is higher than a previous study conducted in Riyadh (13), which is not unexpected because, in the earlier Riyadh study, the researchers focused on children less than five years who were inpatient. In contrast, our study focused on outpatients in healthcare centers and of all age groups.

MERS-CoV co-infection occurred more frequently in patients below 50 years (14.7%) which is consistent with reduced detection of co-infection with increasing age. The MERS-CoV co-infection rate was 25% in patients aged 18-34 years, followed by those aged 35-49 years (9.1%), indicating that increased co-infection of MER-CoV with other viruses was higher among middle-aged people in comparison to children and elderly. It is noteworthy that 33% of the co-infected patients died, which indicates that co-infection with other viruses is a risk factor for death. This high mortality agrees with the previous report by the Saudi Ministry of Health in 2015 of 458 deaths from a total of 1035 confirmed cases of MERS-CoV with a case fatality rate of 44%.

About 83% of the co-infected patients were infected with a single respiratory virus while 1 patient (16.7%) was co-infected with four respiratory viruses. Of all the viruses, HRV was most frequent co-infecter (25%) affecting 2 of the 6 patients, while all the other viruses had equal frequency of co-infection. One patient was co-infected by four viruses (HRV, Cor229, FluA and H1N1). The data show the tendency of HRV to co-infect with MER-CoV but also with other viruses. This observation is not easily explained hence there is need for more research on co-infection of HRV with other respiratory viruses. The only virus found in a female patient was RSV but our data indicates that HRV is the most common virus that co-infect with MER-CoV. The prevalence of co-infection in this study supports previous research of co-infection of MER-CoV with other respiratory viruses.

A major concern of virus co-infection is the tendency for genetic exchange between different viruses through the process of genetic recombination that may result in emergence of more virulent strains of viruses that have increased pathogenicity and enhanced capacity for transmission (15). However, our study was limited by the rather too few numbers of co-infection which is inadequate to support any hypothesis. Nevertheless, our use of advanced molecular diagnostic technique such as the multiplex array aided characterization of the co-infecting respiratory viruses.

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Conflict of interest:

Authors declared no conflict of interest

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**Original Article****Open Access****Infections of implantable cardiac devices by biofilm forming bacteria in western Algeria hospitals**¹Meziani, Z., ¹Hassaine, H., and ²Belhachemi, F.¹Laboratory of Applied Microbiology in Food, Biomedical and Environment (LAMAABE),
University of Tlemcen, Algeria²Department of Cardiology, Hospital of Tlemcen, Algeria*Correspondence to: zahera_fd@yahoo.fr; 00213553406801**Abstract:**

Background: The significant increase in the use of implantable cardiac devices (ICDs) has been accompanied by biofilm formation and increase rate of infection on the devices. The purpose of our study is to describe the clinical and microbiological findings of infection of ICDs in the cardiology units of western Algeria hospitals.

Methodology: All patients with clinical diagnosis of ICD infections or infective endocarditis upon removal of their ICDs from December 2012 to August 2014 in cardiology units of 4 Algerian hospitals were included in the study. Each element of the ICD pocket and lead was separately sonicated in sterile saline, inoculated onto Chapman and MacConkey agar plates and incubated aerobically at 37°C for colony count after 24 hours. Biochemical identification of the bacteria isolates was made by API 20E, API 20 NE and API Staph, and confirmed by Siemens Healthcare Diagnostics WalkAway® 96 Plus System. Antibiotic susceptibility testing on each isolate was performed by the disk diffusion method on Mueller Hinton agar. Biofilm formation was detected by Congo Red Agar (CRA) and Tissue Culture Plate (TCP) methods, and hydrophobicity of the bacterial cell was determined by the MATH protocol.

Results: Over a period of twenty-one months, 17 ICDs were removed from patients with post-operative infections; 6 (35.3%) had early infection of ICD and 11 (64.7%) had late ICD infection. Fifty-four bacterial strains were isolated and identified, with coagulase-negative staphylococci being the predominant bacteria with 46.3% (25/54). There was no significant association between hydrophobicity and antimicrobial resistance in the 54 isolates but there is positive correlation between biofilm production and antimicrobial resistance, with the strongest biofilm producers resistant to more than one antibiotic. Four independent predictors of infection of resynchronization devices were reported; reoperation, multi-morbidity, long procedure, and ICD implantation.

Conclusion: Our study is the first in Algeria to describe microbiological characteristics of ICD infection. The bacteria in the biofilm were protected, more resistant and tolerated high concentrations of antibiotics and thus played a major role in the development of ICD infections. Despite the improvements in ICD design and implantation techniques, ICD infection remains a serious challenge.

Keywords: implantable cardiac devices, staphylococci, resistance, biofilm, hydrophobicity

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Infections des dispositifs cardiaques implantables par des bactéries formant un biofilm dans les hôpitaux de l'ouest Algérien¹Meziani, Z., ¹Hassaine, H., et ²Belhachemi, F.¹Laboratoire de microbiologie appliquée à l'alimentation, la biomédecine et l'environnement (LAMAABE),
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Résumé:

Contexte: L'augmentation significative de l'utilisation des dispositifs cardiaques implantables est un risque majeur d'augmentation du taux d'infection et donc du risque de formation d'un biofilm sur ce genre de dispositifs. L'objectif de notre étude est de décrire les résultats cliniques et microbiologiques de l'infection sur les dispositifs cardiaques implantables (DCI) dans les unités de cardiologie des hôpitaux de l'ouest Algérien.

Méthodologie: Tous les patients cliniquement diagnostiqués avec une infection sur DCI, ou une endocardite infectieuse et ayant subi un retrait de leur dispositif cardiaque sont inclus dans cette étude et cela sur une période entre décembre 2012 et aout 2014 dans 4 unités de cardiologie. Chaque élément du DCI (boîtier et sonde) est trempé séparément dans une solution saline stérile, ensemencé sur deux milieux de culture, un milieu de Chapman et un milieu MacConkey et incubé en aérobiose à 37°C pour la numération des colonies après 24 heures. L'identification biochimique des isolats de bactéries est effectuée par le API 20E, API 20 NE et API Staph, et confirmée par le système WalkAway® 96 Plus de Siemens Healthcare Diagnostics. Les tests de sensibilité aux antibiotiques de chaque isolat sont effectués par la méthode de diffusion des disques sur gélose de Mueller Hinton. La formation d'un biofilm est détectée par les méthodes de la gélose rouge du Congo (CRA) et de la plaque de culture tissulaire (TCP), et l'hydrophobicité de la cellule bactérienne est déterminée par le protocole MATH.

Résultats: Sur une période de 21 mois, 17 DCI sont retirés de patients atteints d'infections postopératoires; 6 patients (35,3%) sont identifiés comme ayant une infection précoce sur leurs DCI et 11 patients (64,7%) ayant une infection tardive. Cinquante-quatre souches bactériennes sont isolées et identifiées, les staphylocoques à coagulase négative étant les bactéries prédominantes avec 46,3% (25/54). Il n'y a pas d'association significative entre l'hydrophobicité et la résistance aux antimicrobiens dans les 54 isolats, mais il existe une corrélation positive entre la production de biofilm et la résistance aux antimicrobiens, les plus puissants en biofilm sont résistant à plus d'un antibiotique. Quatre facteurs prédictifs indépendants d'infection des dispositifs cardiaques implantable sont retrouvés dans ce travail: ré-intervention, longue procédure, sujets multi-tarés, et implantation d'un DCI

Conclusion: Notre étude est la première en Algérie à décrire les caractéristiques microbiologiques de l'infection des DCI. Les bactéries présentes dans le biofilm sont protégées, plus résistantes et tolèrent de fortes concentrations d'antibiotiques et jouent ainsi un rôle majeur dans le développement des infections par DCI. Malgré des améliorations dans les techniques de conception et d'implantation de DCI, l'infection des dispositifs cardiaques implantables reste un problème grave et très couteux.

Mots-clés: dispositifs cardiaques implantables; staphylocoque; résistance; biofilm; hydrophobicité

Introduction:

The permanent implantable cardiac device (ICD) is one of the most widely used cardiac rhythm control devices. Since their introduction in the early 1980s, ICD have become a life-saving therapeutic tool for patients with ventricular arrhythmia. Virtually all the countries surveyed showed significant rise in the use of ICD, the largest implanter being the USA with 434 new implants per million populations (1). Despite improvements in cardiac device design, application of timely infection control practices, and administration of antibiotic prophylaxis at the time of device placement, infections continue to be observed. The infection rate is highly variable, ranging from 0.5 to 12% (2,3).

Infection of implantable cardiac device (ICD) may be local, limited to the pulse generator pocket and/or the subcutaneous portion of the leads, or systemic, involving the transvenous intravascular electrode (4). Several factors have been reported to be associated with a greater risk of ICD infections and these include; immunosuppression (e.g. renal dysfunction and corti-

costeroid use); oral anticoagulation use; co-existing illnesses; periprocedural factors including failure to administer perioperative antimicrobial prophylaxis; device revision/replacement; and operators' experience (5).

Because of the invasiveness, these devices have multiple comorbidities in ICD recipients, the benefits of which can be overshadowed by infectious complications. Infection of ICD is usually a serious and fearsome complication requiring both complete removal of the infected device and systemic antimicrobial treatment (5,6). Although the implantation of pacemakers is a procedure characterized by low rate of complication, infections, mainly of the pacemaker generator pocket, is one of the most common complications with incidence ranging from 1 to 5%. Despite the low incidence, such infections have a worrying development, mostly because it is associated with high morbidity and potential fatality (7).

Microbiological diagnosis of ICD infection is of great importance for appropriate treatment. Staphylococcal species including *Staphylococcus aureus* and coagulase negative staphylococci (CoNS) represent the

majority of bacterial agents of ICD infection (8,9). However, some unusual microorganisms such as *Propionibacterium* spp., *Corynebacterium* spp., *Acinetobacter baumannii*, and *Haemophilus influenzae* may also be involved and antibiotic resistance is often detected in them (10,11). Furthermore, one major concern among professionals and bacteriologists is the risk of biofilm formation on the ICDs which consequently favors infection. In recent decades, biofilm formations have been widely reported to be responsible for nosocomial infections especially of prosthetic implants, tubes, leads and catheters (12,13).

It is speculated that the presence of an ICD is conducive for microbial colonization contributing greatly to the development of biofilms, which in turn, explains the occurrence of endocarditis (14,15). It is interesting to note that biofilm consists of a structured medium of microbial cells that adhere to a solid surface and is surrounded by a matrix of extracellular polymeric substances. This microbial combination is a form of protection encouraging symbiotic relationships, tolerance, as well as antimicrobial resistance (16).

The goal of this study is to first describe the bacteriological characteristics of ICD infections over a period of 2 years in four cardiology units of one private and three university hospital centers in western Algeria, and secondly to determine whether the isolated bacteria have the ability to form biofilms that may be responsible for triggering infection, especially in late stage endocarditis.

Materials and method:

Study setting:

This study is carried out at the Laboratory of Microbiology Applied to Food, Biomedical and Environment (LAMAABE) of the University Abou-Bekr Bekaid-Tlemcen, Algeria.

Subjects

All the patients with clinical diagnosis of ICD infection or infective endocarditis upon removal of their cardiac devices from December 2012 to August 2014 in four cardiology units of three hospitals in Algeria were included in this study. The clinical diagnosis of ICD infection was made by the clinician based on local signs of inflammation such as erythema, heat, redness or purulent drainage, outward displacement of the pocket, septicemia or infectious endocarditis of the heart. The time of infection was recorded and ICD infection was categorized

as; 'very early' if infection occurs 1 month after the latest ICD procedure; 'early' if infection occur between 2 and 12 months of the latest procedure, and 'late' if infection occurs 12 months after the latest procedure.

Ethical approval

Ethical clearance was obtained from the Institutional Review Board at the Faculty of Health Science of the University of Algeria. The administrative authorizations were obtained from the Delegation of Public Health of the Regional Hospitals of Western Algeria. Moreover, written informed consent was obtained from all study patients prior to interview and DCI collection

Collection of demographic and clinical data

Demographic and clinical data collected from each patient by direct interview included age, gender, existence of an underlying anterior cardiopathy or a history of infective endocarditis, existence of diabetes, immunosuppression, primary implantation or change of pocket, antibiotic therapy and possible infection or surgery programmed during the last five years.

Microbiological culture of the ICDs

After removal by the surgeons, the implantable cardiac devices (ICDs) were placed in sterile boxes and transported to the microbiology laboratory within 30 minutes. Each element of the ICD pocket and lead was soaked separately in sterile saline solution and sonicated for 5 minutes at a frequency of 20Khz, and vortexed for 30sec to detach and detect sessile bacteria hanging on to these devices (17,18). After sonication, 100µL of the soaking liquid was inoculated onto Chapman agar and MacConkey agar plates. The number of CFU/mL was counted after 24 hours aerobic incubation. The minimum detection threshold was 2 CFU/mL (19).

Biochemical identification and susceptibility testing of bacteria isolates

The biochemical identification of the bacteria isolates was done using the API 20E and API 20NE Staph API (BioMérieux, Marcy l'Etoile, France), and confirmed by the Siemens Healthcare Diagnostics WalkAway® 96 Plus System. The antibiotic susceptibility and resistance phenotypes were determined by the disk diffusion method on Mueller-Hinton agar (Biorad, Marnes-la-Coquette, France) according to the recommendations of the antibiogram committee of the French Society of Microbiology (20). The antibiotic disks used were penicillin (10iu), oxacillin (5µg), cefoxitin (30µg), gentamicin (10µg),

tobramycin (10µg), amikacin (30µg), vancomycin (30µg), rifampin (30µg), fosfomycin (50µg), fusidic acid (10µg), clindamycin (2 µg), pristinamycin (15µg), erythromycin (15 µg), ofloxacin (5µg), tetracycline (30µg), chloramphenicol (30µg), imipenem (10µg), and trimethoprim/sulfamethoxazole (25µg), cefotaxime (5µg), ceftriaxone (30µg), nalidixic acid (30µg), ciprofloxacin (5µg), amoxicillin (20µg), and clavulanic acid (10µg).

Detection of biofilm by Tissue Culture Plate (TCP) method

Quantitative measurement of biofilm production in the isolates was done using a microtiter assay. In brief, cells were grown overnight in Brain Heart Infusion Broth (BHIB). The broth was then diluted 1:100 and inoculated into microtiter plates. After 24 hours incubation at 37°C, the plates were washed, stained with crystal violet, and the optical density measured at 570 nm (20). Each test was performed in triplicate. As a negative control, the uninoculated medium was used to determine the initial OD. Mean OD values were calculated for all tested strains and the negative control.

The threshold value (ODc) was established as three standard deviations (SD) above the mean OD of the negative control i. e. $ODc = \text{mean OD of the negative control} + (3 \times SD \text{ of negative control})$. The ODc value was calculated separately for each microtiter plate. When a negative value was obtained, it was presented as zero, while any positive value indicated the production of biofilms. For the interpretation of the results, the classification of the obtained results was based on the control OD. The strains are classified as follows: $OD \leq DOT$ (control) is non-biofilm forming; $DOT \times 2 \leq OD \leq DOT \times 4$ is moderate biofilm-forming, and $DOT \times 4 \leq OD$ is highly (strong) biofilm-forming (21).

Detection of biofilm by Congo Red Agar (CRA) method

The Congo Red Agar method was used for detecting the ability of isolate to produce slime. The CRA medium was prepared with 37 g/L BHI broth, 50 g/L sucrose, 10 g/L agar, and 0.8 g/L Congo Red. The Congo Red stain was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 min separately from other medium constituents, and was then added when the agar had cooled to 55°C. The plates were inoculated with bacterial isolate and incubated at 37°C for 24 hours. A positive result was indicated by black colonies while non-producing strains developed red colonies (22). For the evaluation of colonies colors, a

four-color reference scale was used according to Satorres and Alcaráz (23). Black and almost black burgundy were classified as biofilm producers, while burgundy and red as non-biofilm producing strains. This test was performed in triplicate

Hydrophobicity assays

The hydrophobicity of the bacterial wall was evaluated with the MATH protocol (24) using hexadecane as a solvent. The bacteria strains were first grown in 50 mL of Luria Bertani (LB) and incubated for 18 hours at 37°C. The cells were recuperated by centrifugation at 5000 rpm for 15 min. The pellet obtained was then washed after two successive centrifugations with PBS (Phosphate Buffered Saline pH 7.1) and suspended in the same buffer at an initial optical density (ODi) between 0.8 and 1 at 600nm. A volume of 0.3ml of each solvent was added to 1.8ml of bacterial suspension and the mixture was vortexed for 2 min. After a 20 min settling, the optical density (ODf) of the aqueous phase was measured at 600nm and the percentage of adhesion to solvent was then calculated using the following equation; $CSH \% = [(ODi - ODf) / ODi] \times 100$ (25). A CSH of 0-20% is defined as weak, 21-50% as moderate and > 40% as strong hydrophobicity.

Results:

From December 2012 to August 2014, a total of 315 implantable cardiac devices (ICDs) were implanted by the cardiology units of the four hospitals, out of which ICD was removed in 17 patients, all of whom developed infection on the ICDs, giving an infection rate of 5.4%. All 17 patients underwent only one surgical procedure except for one patient who had two surgical procedures within three months due to repeated infections of his ICD. The age range of the patients was 60 to 75 years; 13 (76.4%) were male and 5 (29.4%) were diabetic.

All the patients were on antibiotic therapy on the day of cardiac device insertion. Four independent predictive factors of ICD infection identified were; re-intervention, long procedure, multi-morbidity, and ICD implantation procedure. Six patients (35.3%) had early infection of ICD while 11 patients (64.7%) had late infection. There were 9 patients with associated primary ICD implantation infections while there were 8 with change of pocket ICD infection (Table 1).

Table 1: Distribution of bacteria strains according to the implantable cardiac device (ICD)

Pacemaker	Isolated strains		Hospital	Gender (M/F)	Duration of the intervention	Primo or change of pocket (P/C)	Endocarditis
	Pocket	Lead					
P1	Negative	S1: <i>Moraxella</i> S'1: <i>Staphylococcus xylosum</i>	CHU Tlemcen	F	50min	P	
P2	P'2: <i>Staphylococcus xylosum</i>	Negative	CHU Tlemcen	M	1h 20 min	C	
P3 (DAI)	P3: <i>Pseudomonas aeruginosa</i>	S3: <i>Enterobacter cloacae</i> S3p: <i>Pseudomonas aeruginosa</i>	EH Ain Temouchent	M	7h	P	
P4	P'4: <i>Staphylococcus épidermidis</i>	S'4: <i>Staphylococcus épidermidis</i>	EH Ain Temouchent	M	4hs	P	
P5	P'5: <i>Staphylococcus sciuri</i>	S5: <i>Proteus penneri</i>	CHU Tlemcen	M	1H30 min	P	
P6	P6: <i>Pseudomonas aeruginosa</i> P'6: <i>Staphylococcus sciuri</i>	S6: <i>Vibrio fluvialis</i> S'6: <i>Staphylococcus capitis</i>	CHU Tlemcen	F	1h 10 min	C	Positive
P7	P'7: <i>Staphylococcus sciuri</i>	S'7: <i>Staphylococcus sciuri</i>	CHU Tlemcen	M	25 min	C	
	P8: <i>Vibrio fluvialis</i>	S8: <i>Vibrio vulgaris</i>	CHU Tlemcen	M	1h 15 min	P	
P8	P'8: <i>Staphylococcus sciuri</i>	S'8: <i>Staphylococcus épidermidis</i>					
P9	P9: <i>Pseudomonas horzihabitans</i> P'9: <i>Staphylococcus simulans</i>	S9: <i>Pseudomonas aeruginosa</i> S'9: <i>Staphylococcus haemolyticus</i>	EH Ain Temouchent	M	20 min	P	
	P10: <i>Proteus penneri</i>	S10: <i>Proteus penneri</i>	CHU Tlemcen	F	1h 10 min	C	
P10	P''10: <i>Chryso bacterium indolegenes</i> P'10: <i>Staphylococcus hominis</i>	S'10: <i>Staphylococcus warneri</i>					
P11	P11 : <i>Pasteurella pneumotropica</i> P'11: <i>Staphylococcus lugdunensis</i>	S11: <i>Ochrabactrum antropi</i> S'11: <i>Staphylococcus épidermidis</i>	CHU Tlemcen	M	1h 15min	C	
P12	P12: <i>Ewingella americana</i> P'12: <i>Staphylococcus epidermidis</i>	S12: <i>Ewingella americana</i> S'12: <i>Staphylococcus epidermidis</i>	CHU Tlemcen	M	1h	P	
P13	P13: <i>Pseudomonas aeruginosa</i> P'13: <i>Staphylococcus hominis</i>	S13: <i>Vibrio fluvialis</i>	CHU Tlemcen	M	20 min	C	Positive
P14	P14: <i>Photobacterium damsela</i>	S14: <i>Photobacterium damsela</i>	CHU Tlemcen	M	25min	C	
P15	P15: <i>Serratia liquefaciens</i>	S15: <i>Enterobacter aerogenes</i> S'15: <i>Staphylococcus épidermidis</i>	Kara Clinic	M	1h	C	Positive
P16	P16: <i>Serratia plymuthica</i> P'16: <i>Pasteurella pneumotropica</i> P'16: <i>Staphylococcus hominis</i>	S16: <i>Photobacterium damsela</i> S'16: <i>Staphylococcus hominis</i>	CHU Tlemcen	F	55min	P	Positive
P17	P17: <i>Photobacterium damsela</i> P'17: <i>Staphylococcus épidermidis</i>	S17: <i>Moraxella</i> S'17: <i>Staphylococcus épidermidis</i>	CHU Tlemcen	H	1h 15min	P	

CHU: University Hospital Centre

EH: Hospital / Health Institution

Kara clinic: Private Clinic

All 17 ICDs (pockets and leads) were positive for bacteria on culture plates, and 54 bacteria (26 Gram positives and 26 Gram-negatives) were isolated and identified, with the most frequent being coagulase-negative staphylococci (CoNS) representing 46.3% (25

/54) of the total isolates (Table 2 and 3). A total of 27 bacterial isolates were recovered from the ICD pockets and 27 from the ICD leads. The most frequently isolated enteric bacteria were *Pseudomonas* spp, *Klebsiella* spp, *Proteus* spp., *Serratia* spp, and *Entero-*

bacter spp identified in 9 ICDs comprising 29 out of the 54 (53.7%) strains.

Other infrequently isolated bacteria from some ICDs included *Ochrabactrum antropi*, *Ewingella americana*, and *Photobacterium damsela* which were not associated with infectious endocarditis in the patients. Polymicrobial infection was recorded in 15 of the 17 patients (88.2%) with varying

combinations of *Pseudomonas aeruginosa*, *Staphylococcus sciuri*, *Vibrio fluvialis* and *Staphylococcus capitis* while the infection was monomicrobial in a patient (P₄) where the ICD pocket and the lead were colonized by the same *Staphylococcus epidermidis* strain and another patient (P₂) where only the pocket was colonized by *Staphylococcus xylosum* (Table 1).

Table 2: Distribution of Gram-negative bacteria strains according to the implantable cardiac device (ICD)

Strains	Production of slime	Tissue culture plate	MATH	Antibiotype
S1 <i>Moraxella</i>	Non- Producer	Strong		na ^R amp ^R , amc ^R cro ^R ctx ^R kf ^R
P3 <i>Pseudomonas aeruginosa</i>	Non- Producer	Strong	Strong	caz ^R ctx ^R , tcc ^R cn ^I
S3 <i>Enterobacter cloacae</i>	Producer	Strong	Strong	cn ^R c ^R ak ^I ffi ^R cro ^R te ^R amc ^R tob ^R fa ^R ctx ^R amp ^R
S3p <i>Pseudomonas aeruginosa</i>	Producer	Strong	Strong	tob ^I na ^R cn ^R te ^R amc ^R cro ^R kf ^R amp ^R ctx ^R caz ^R
S5 <i>Proteus penneri</i>	Producer	Strong		amc ^R amp ^R kf ^R ipm ^R ctx ^R cro ^R ofx ^R cip ^R na ^R cn ^R tob ^R te ^R
P6 <i>Pseudomonas aeruginosa</i>	Producer	Strong	Weak	tob ^R ak ^I na ^R cn ^R te ^R amc ^R cro ^R kf ^R amp ^R ctx ^R caz ^R
S6 <i>Vibrio fluvialis</i>	Producer	Strong		na ^R te ^I amc ^R cro ^I kf ^R amp ^R ctx ^R
P8 <i>Vibrio fluvialis</i>	Producer	Strong		na ^R amc ^R kf ^R amp ^R ctx ^R
S8 <i>Vibrio vulgaris</i>	Producer	Strong		ak ^R na ^R cn ^R te ^R amc ^R cro ^R kf ^R amp ^R ctx ^R caz ^R
P9 <i>Pseudomonas horyzihabitans</i>	Producer	Strong	Weak	tob ^R ak ^R na ^R cn ^R te ^R amc ^R cro ^R kf ^R amp ^R ctx ^R
S9 <i>Pseudomonas aeruginosa</i>	Producer	Strong	Moderate	cip ^R ofx ^R tob ^R ak ^R na ^R cn ^R te ^R amc ^R cro ^R kf ^R amp ^R ctx ^R
P10 <i>Proteus penneri</i>	Producer	Strong		cip ^R ofx ^R na ^R ctx ^I
P ¹⁰ <i>Chrysobacterium indolegenes</i>	Producer	Strong		cip ^R ofx ^R tob ^I na ^R cn ^I te ^R amc ^I kf ^R ctx ^R
S10 <i>Proteus penneri</i>	Producer	Strong		na ^I amc ^R cro ^R kf ^R amp ^R ctx ^R
P11 <i>Pasteurella pneumotropica</i>	Producer	Strong		na ^R amc ^R kf ^R amp ^R ctx ^R cro ^R
S11 <i>Ochrabactrum antropi</i>	Producer	Strong	Weak	na ^R amc ^R kf ^R amp ^R ctx ^R ipm ^R
P12 <i>Ewingella americana</i>	Producer	Strong	Strong	ctx ^R cro ^R amp ^R fa ^I amc ^R tob ^R
S12 <i>Ewingella Americana</i>	Producer	Strong	Strong	ctx ^R cro ^R amp ^R fa ^I amc ^R tob ^R
P13 <i>Pseudomonas aeruginosa</i>	Producer	Strong	Strong	ctx ^R cro ^R amp ^R c ^R fa ^R caz ^R ffi ^R te ^R amc ^R tob ^I
S13 <i>Vibrio fluvialis</i>	Non- Producer	Strong		ctx ^R cro ^R amp ^R fa ^I cip ^I ffi ^R ofx ^I te ^R amc ^R tob ^R ipm ^R
P14 <i>Photobacterium damsela</i>	Producer	Strong	Weak	ctx ^R cro ^R amp ^R te ^R amc ^R
S14 <i>Photobacterium damsela</i>	Producer	Strong	Weak	ctx ^R cro ^R amp ^R te ^R amc ^R
P15 <i>Serratia liquefaciens</i>	Producer	Strong	Strong	ctx ^R cro ^R amp ^R fa ^R ffi ^R te ^R amc ^R tob ^R
S15 <i>Enterobacter aerogenes</i>	Producer	Strong	Strong	ctx ^R cro ^R amp ^R cn ^R kf ^R te ^R amc ^R tob ^R caz ^R
P16 <i>Serratia plymuthica</i>	Non- Producer	Strong	Strong	amc ^I cro ^R kf ^R amp ^R ctx ^R
S16 <i>Photobacterium damsela</i>	Producer	Strong	Weak	cn ^I tob ^I amc ^R cro ^R amp ^R ctx ^R
P17 <i>Photobacterium damsela</i>	Producer	Strong	Weak	tob ^I cro ^I
S17 <i>Moraxella</i>	Non- Producer	Strong	Strong	ctx ^R cro ^R amp ^R te ^I amc ^R
P ¹⁶ <i>Pasteurella pneumotropica</i>	Non- Producer	Weak		cn ^I tob ^I amc ^R cro ^R kf ^R amp ^R ctx ^R

na=nalidixic acid; amp=ampicillin; amc=amoxicillin; cro=ceftriaxone; ctx=cefotaxime; caz=ceftazidime; te=tetracycline; tob=tobramycin; fa=fusidic acid; ffi=fosfomicin; ipm=imipenem; cip=ciprofloxacin; ofx=ofloxacin; cn=chloramphenicol

Table 3: Distribution of Gram-positive bacteria strains according to the implantable cardiac device (ICD)

Strains	Production of slime	Tissue culture plate	Math	Antibiotype
P'2 <i>Staphylococcus xylosum</i>	Non- Producer	Strong		na ^l k ^R va ^l
P'9 <i>Staphylococcus simulans</i>	Producer	Strong	Strong	na ^R va ^l te ^R
P'10 <i>Chrysobacterium indolegenes</i>	Producer	Strong		cip ^R ofx ^R tob ^l na ^R cn ^l te ^R amc ^l kf ^R ctx ^R
S'1 <i>Staphylococcus xylosum</i>	Producer	Moderate	Weak	na ^l k ^R va ^l
P'4 <i>Staphylococcus epidermidis</i>	Non- Producer	Moderate	Strong	ox ^R tob ^R cn ^R va ^l ox ^R
S'4 <i>Staphylococcus epidermidis</i>	Non- Producer	Moderate		ox ^R ra ^S tob ^R cn ^R va ^l ox ^R
P'5 <i>Staphylococcus sciuri</i>	Producer	Moderate	Strong; Hydrophobe	na ^R amc ^R cro ^l amp ^R ctx ^R kf ^R va ^l
S'6 <i>Staphylococcus capitis</i>	Producer	Moderate	Moderate	na ^R amc ^R cro ^l amp ^R ctx ^R kf ^R va ^l
P'7 <i>Staphylococcus sciuri</i>	Producer	Moderate	Weak	na ^l c ^l va ^l te ^R
S'7 <i>Staphylococcus sciuri</i>	Producer	Moderate	Weak	na ^l c ^l va ^l te ^R
S'8 <i>Staphylococcus epidermidis</i>	Producer	Moderate	Weak	ox ^l na ^R c ^R va ^l te ^R tob ^l
P'10 <i>Staphylococcus hominis</i>	Producer	Moderate	Strong; Hydrophobe	cro ^l kf ^l ctx ^R
S'10 <i>Staphylococcus warneri</i>	Producer	Moderate	Weak	na ^l amc ^R cro ^l kf ^R amp ^R ctx ^R ff ^R
P'11 <i>Staphylococcus lugdunensis</i>	Producer	Moderate	Weak	na ^l kf ^R amp ^l ctx ^R
S'11 <i>Staphylococcus epidermidis</i>	Producer	Moderate	Strong	na ^l amc ^R cro ^R kf ^R amp ^R ctx ^R
P'12 <i>Staphylococcus epidermidis</i>	Producer	Moderate	Weak	ox ^R na ^R n ^l va ^l te ^R tob ^R
P'13 <i>Staphylococcus hominis</i>	Non- Producer	Moderate	Weak	va ^l te ^R
S'15 <i>Staphylococcus epidermidis</i>	Producer	Moderate	Weak	ox ^R na ^S pnv ^R n ^S c ^S k ^S cip ^S pef ^S va ^l te ^R ra ^R tob ^R
P'16 <i>Staphylococcus hominis</i>	Producer	Moderate	Weak	na ^l va ^l
S'16 <i>Staphylococcus hominis</i>	Producer	Moderate		ox ^l na ^R n ^l va ^l te ^R
P'17 <i>Staphylococcus warneri</i>	Producer	Moderate	Strong	na ^l va ^l
P'6 <i>Staphylococcus sciuri</i>	Producer	Weak	Weak	na ^R va ^l
P'8 <i>Staphylococcus sciuri</i>	Producer	Weak	Weak	tob ^l ak ^l na ^R cn ^R te ^S amc ^R cro ^R kf ^R amp ^R ctx ^R
S'9 <i>Staphylococcus haemolyticus</i>	Producer	Weak	Strong; Hydrophobe	na ^R amc ^R cro ^R kf ^R amp ^R ctx ^R
S'12 <i>Staphylococcus chromogenes</i>	Producer	Weak	Weak	na ^l k ^R va ^l te ^l tob ^R
S'17 <i>Staphylococcus epidermidis</i>	Producer	Weak	Weak	na ^l k ^R va ^l tob ^R

na=nalidixic acid; tob=tobramycin; va=vancomycin; cn=chloramphenicol; te=tetracycline; cro=ceftriaxone; ctx=cefotaxime; amp=ampicillin; amc=amoxicillin; ox=oxacillin; ff=fosfomycin; cip=ciprofloxacin; pnv=pristinamycin; pef=pefloxacin

This study of antibiotic susceptibility of the isolated strains in the four hospital centers showed resistance at a significant level against several antibiotics (Fig 1 and Fig 2). Fig 1 is a histogram illustrating the resistant pattern of the Gram-negative bacterial isolates showing high resistance rate (>90%)

to amoxicillin, clavulanic acid, cefotaxime, and gentamycin, with 3 isolates resistant to imipenem. None of the staphylococcal strains was resistant to vancomycin. Fig 2 is a histogram illustrating the resistance pattern of the Gram-positive isolates.

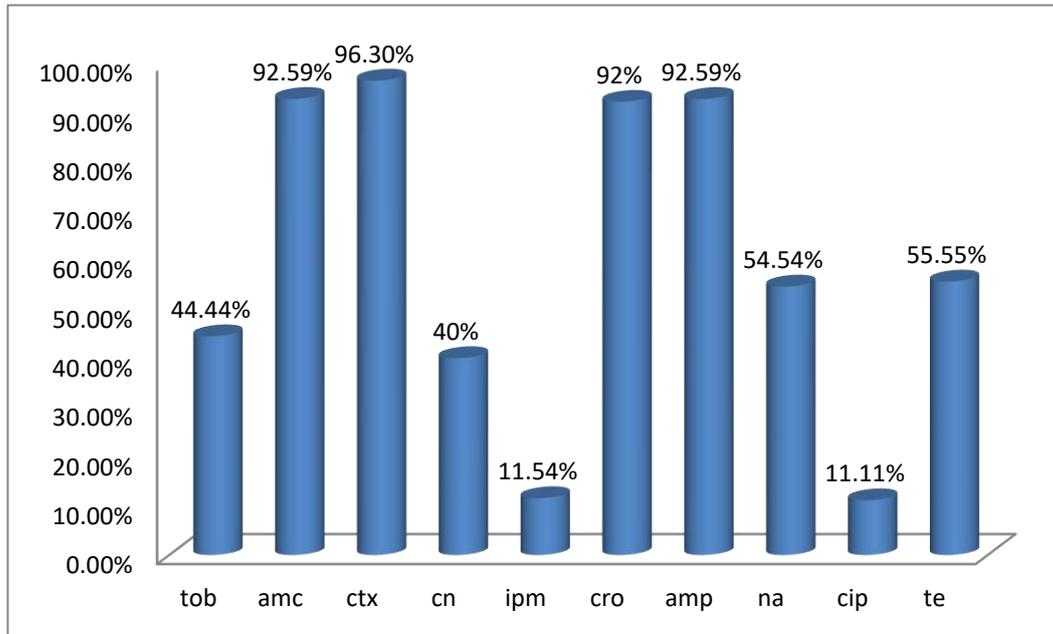


Fig 1: (Histogram A₁): Resistance rate of Gram-negative bacteria isolates

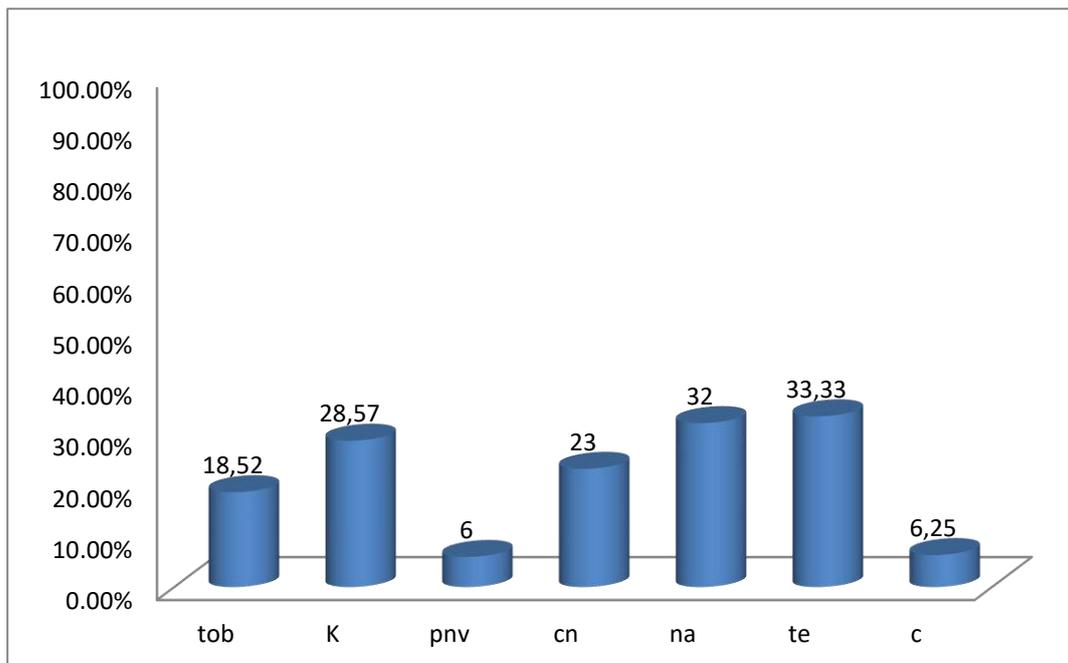


Fig 2: (Histogram A₂): Resistance rate of Gram-positive bacteria isolates

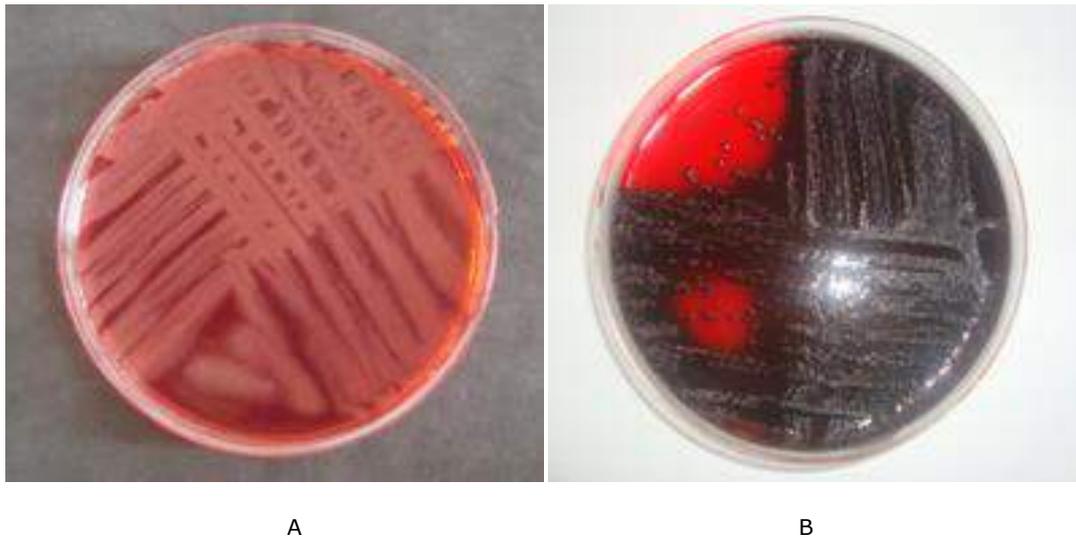


Fig 3: Slime production in the strains isolated on Congo red medium
 A: slime generating, B: non-slime generating

Results of biofilm detection

The results of the biofilm production by the bacterial isolates with the qualitative CRA and the quantitative TCP methods are presented in Table 2. Out of the 54 bacteria isolates, 44 (81.5%) were slime producers by the CRA method and 24 of them were strong (very good) biofilm producers using the TCP method, with OD 570 > 0.240. The TCP method detected biofilms in 48 (88.9%) of 54 isolates with different intensities; 30 (55%) isolates were strong producers, 18 (33.3%) isolates were moderate, 6 (11.1%) isolates were low biofilm producers, while 10 (18.5%) were non-producers of biofilms. Thirty-seven (68.5%) of the 54 isolates were positive by both TCP and RCA methods. Fig 3 is a photograph of CRA plate showing slime prod-

uction by a positive isolate (A) and non-slime production by a negative isolate (B).

Result of hydrophobicity

The results of the microbial adhesion to the solvent (hydrophobicity) are summarized in Figs 4 and 5. A total of 14 Gram positive and 7 Gram negative bacteria isolates had affinity to hexadecane (apolar solvent) suggesting a weak hydrophilic character. On the other hand, 17 (41.5%) isolates were relatively hydrophobic between 1% and 96%, and 2 (5%) strains were moderately hydrophobic. Fig 4 is a histogram of the cell surface hydrophobicity of the Gram-negative bacteria isolates and Fig 5 is a histogram of the cell surface hydrophobicity of Gram-positive bacteria isolates.

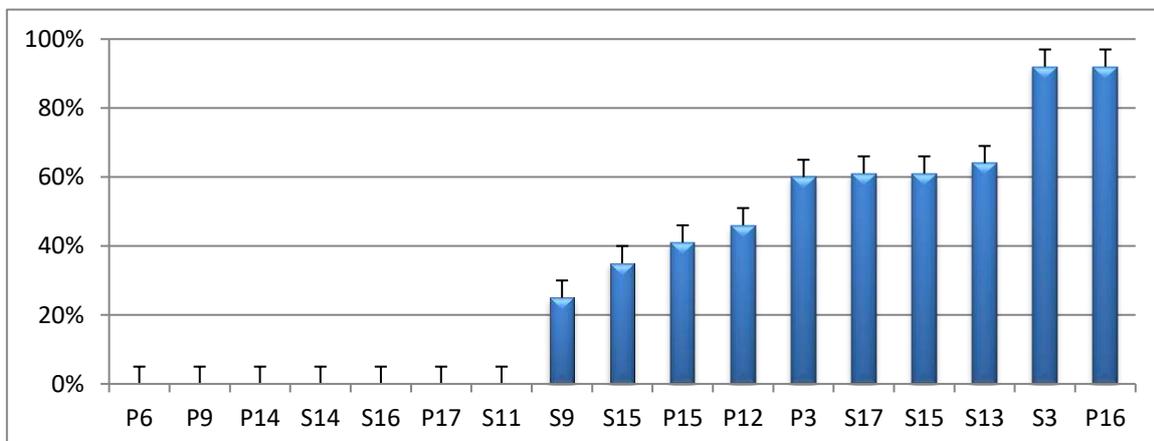


Fig 4: (Histogram B₁): Cell surface hydrophobicity of Gram-negative bacteria isolates

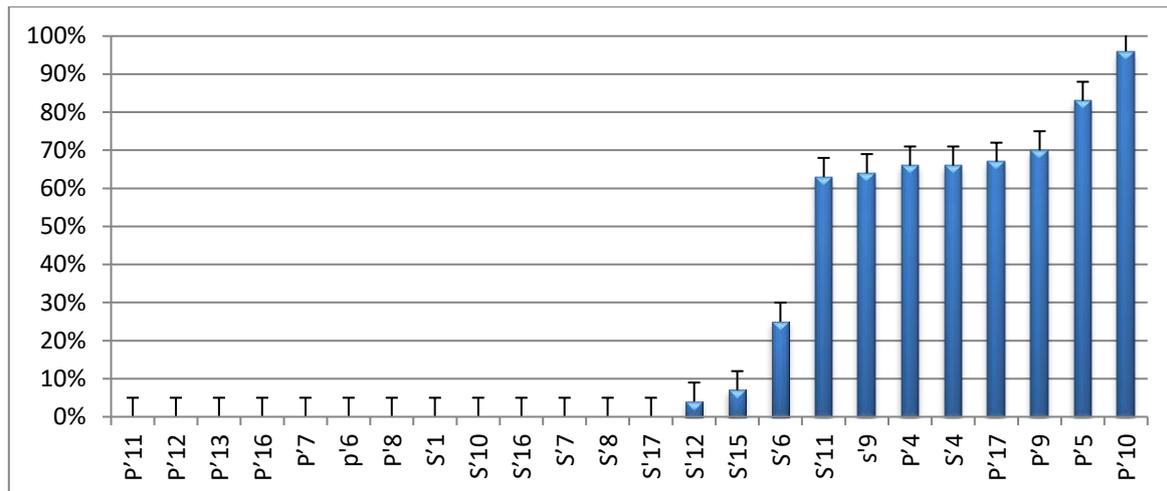


Fig 5: (Histogram B₂): Cell surface hydrophobicity of Gram-positive bacteria isolates

Discussion:

ICDs have become increasingly of great importance in the management of heart disease in many countries around the world with a great impact on the quality of life of patients (10). The advances in the development of permanent pacemakers and the technologies of implantable cardiac defibrillators have helped this process. Nevertheless, with the increase in ICD implantations, infections on these devices have correspondingly increased. To the best of our knowledge, this is the first and only study on the microbiological characteristics of ICD infections in Algeria. Although our data reflect the epidemiology of ICD infections in the region, they could be of great interest and provide useful information for ICD infections management in other regions.

In recent decades, the rate of these ICD infections has increased worldwide from 0.13% to 19.9% with an average rate of 10% (15,26). The overall incidence of infection on ICD implantable cardiac devices is estimated at 1.9 per thousand per year (27). According to an estimate by Camus et al., there are more than three million patients on pacemaker (PM) worldwide and 180,000 carriers of implantable defibrillator (ID) (28). Among the complications occurring after the implantation of an ICD, infection is certainly the most serious (29). In this retrospective study of 21 months, of a total of 315 ICD implantations in 4 hospitals, 17 patients presented with infection of their ICDs, giving an incidence rate of 5.4%. All the infections were diagnosed and confirmed by specialist cardiologists with clinical presentations that

included erythema, heat, redness with or without purulent drainage, sepsis and infectious endocarditis. This rate is quite high when compared to other studies such as the French study by Klug and his team (30) in 2000 where the incidence of infectious complications was only 0.68%. In 2010, Baddour et al., (31) and Voigt et al., (32) estimated the incidence to be between 0.13% and 20%, which was related to the increase in the implantation rate of ICDs in the world in general. Of the 17 infected patients, 16 had infection of their pacemakers (PM), giving an incidence rate of 5.1% in the study. In the literature, the incidence of pacemaker infections is known to vary between 0.13% and 19.9% occurring mostly at the site of implantation of the generator, and in 10% of cases, it is the cause of infectious endocarditis (33). Only one patient had an infection after defibrillator implantation (DI) representing an incidence of 0.3%, which is known to vary between 0.7% and 1.2% (34).

The infection of an ICD pocket is a rare but much feared early complication, which is defined as an infection occurring within the six months following the procedures (35) and often accompanied by fever and local signs that require urgent antibiotic therapy and immediate removal of the device (36). In our study, 6 of the 17 (35.3%) infected patients had early infection of ICD. According to the study by Ben Abid et al., (37), 25% of ICD infections are early and 42% are late, with no significant difference reported with respect to the type of infectious organisms. Nine of the 17 (52.9%) infected patients had an outward displacement of the pocket, a rate that is considerably higher

compared to the study of Klug et al., (38) where 29.5% of the patients presented with an outward displacement of the pocket in a study of 105 patients.

There are various factors predisposing to ICD infection that have been reported including advanced age, diabetes, cancer and immunosuppression, anticoagulation, presence of a temporary stimulation lead, duration of intervention, and the surgeon's experience (39). Indeed, diabetes mellitus and age are predisposing factors in most of our patients. The highest rate of infection was observed in the age group 60 to 75 years who were mainly male patients among whom 29.4% were diabetic. A total of 15 patients had positive cultures on the lead and the pocket of their ICDs, 8 of whom were infected by the same bacterium. Victor et al., (40) reported that the infection of the lead is associated with that of the pocket in every two cases. This infection can occur in isolation and in one fourth of cases a few weeks after implantation (40). The culture of the removed devices allows the possibility of an etiological diagnosis of ICD infection in the vast majority of cases. This means that microbiological analysis is mandatory in the case of suspicion of an ICD infection.

A total of 54 bacteria were isolated and identified from the ICDs; 46.3% Gram positive bacteria were isolated, which were mostly CoNS with predominance of *S. epidermidis*. These findings closely agree with those reported by Gill et al., (41) who estimated that about 41.2% of ICD infections were caused by CoNS. The dominant role of staphylococci is consistent with what has been previously reported where staphylococcal species accounted for 60 to 80% of the cases in most of the reported studies (36,42,44-44). In several studies, *S. epidermidis* was the most frequently isolated CoNS species and constitutes a significant part of the normal bacterial flora of human skin and mucous membranes from where it is easily introduced as a contaminant during surgical implantation of the polymer device (45).

Gram-negative bacteria constituted 53.7% of the isolated bacteria, with high frequency of enteric bacteria such as *Proteus* spp, *Serratia* spp, *Enterobacter* spp and *Pseudomonas* spp. These bacteria which are involved contamination and care-related infections were recovered twice in the space of a few weeks in two different hospitals. This implies either contamination or poor technique during the surgical procedure. We noted the exceptional recovery of three uncommon bacteria species; *Ewingella ameri-*

cana, *Ochrabactrum antropi*, and *Photobacterium damsela* from the ICDs in our study, which have been reported only by a few authors such as Pien et al., (46), Mahmood et al., (47), and Austin et al., (48). Nevertheless, the important causative microorganisms of ICD infections are CoNS followed by Gram-negative bacilli, fungi, non-tuberculous mycobacteria and *Bulkholderia cepacia*. The bacterial complex is a rare cause of ICD infection with only a few cases of ICD pocket infection reported in the literature (41,49,50-51). ICD infections are mainly caused by the contamination of the local bacterial flora during implantation. Skin micro-organisms migrate from the insertion site along surface of the lead, colonize the intravascular distal portion, and finally infect the blood (52).

Nearly all of the isolated Gram-negative bacteria in this study were resistant to ampicillin, amoxicillin, cefotaxime, and ceftriaxone. Although imipenem was highly active on the tested strains, three isolates were resistant to this antibiotic. These strains were also resistant to quinolones with rates ranging from 11.1% for ciprofloxacin to 54.5% for nalidixic acid. Resistance rates of isolates to tetracycline and tobramycin were 55.5% and 44.4% respectively. Unlike Gram-negative bacteria, CoNS isolates displayed a higher level of sensitivity but with some were resistant to nalidixic acid and tetracycline. According to 2008 Camus study (28), majority of staphylococci are multi-sensitive with especial sensitivity to aminoglycosides, but the isolates in our study were resistant to kanamycin (28.5%) and tobramycin (18.5%).

The development and persistence of bacterial infections are often associated with the foreign equipment and materials used in the devices and the ability of these bacteria to adhere to them. The bacterial strains implanted on the surface of a device are inserted and protected by a dense polysaccharide extracellular matrix and are more resistant to antibiotics, constituting the biofilm that can be detected by quantitative and qualitative techniques (1). Adherence and persistence of CoNS infection are often associated with foreign materials. Adhesion is primarily related to non-specific physico-chemical forces, and then to specific interaction of the bacterial surface adhesins (not directly with the device) with host proteins coating the device (53). CoNS may adhere directly to the device plastic polymers via fimbria-like surface protein structures or via a capsular polysaccharidic adhesion (54). Bacteria may also adhere to host matrix proteins such as fibrinogen, fibronectin, and

collagen that coat the surface of an implanted device (55). The layers of bacteria on the surface of an implanted device are encased in an extracellular slime made of a polysaccharide intercellular adhesion and constitute the biofilm (56,57). Microbes in a biofilm are protected by this dense extracellular matrix and are more resistant to antibiotics and host defenses. Biofilm forming bacteria are therefore a public health challenge for those requiring indwelling medical devices (56).

In our study, the presence of infection was influenced by the ability of 48 out of 54 strains to form a biofilm, whatever the support (pocket and leads), and thus representing a significant risk factor. The TCP technique, which is the most widely used method, is considered the 'gold standard' test for sensitive, accurate and reproducible screening method for the detection of biofilm production in clinical isolates (58). The adherence capacity of clinical isolates to host cells depends on bacterial surface properties such as hydrophobicity. In a recent study, it was reported that there is a significant difference in cell surface load between group B streptococci treated with antibiotics and those untreated (59). The determination of the acid-base properties (acceptor electron, donor electron) may be of great importance in many research areas of phagocytosis and microbial adhesion (60).

Our data indicated that affinity to hexadecane (apolar solvent) suggests low hydrophilicity for the majority of the bacteria isolated from surfaces of hydrophobic medical device. This contradicts several studies which reported that hydrophobic cells tend to adhere to hydrophobic substrates, while hydrophilic cells tend to adhere to hydrophilic substrates (61,62). It has also been suggested that there is a positive correlation between the degree of bacterial hydrophobicity and adhesion to abiotic surfaces (63). Our finding can be explained by the fact that these bacteria adhere to host proteins such as fibrinogen, fibronectin, and collagen which cover the surface of the implanted device, making it hydrophobic (55). The comparison between the biofilm formation (by RCA and TCP), hydrophobicity and antimicrobial resistance of the 54 bacteria isolates revealed that there is no significant association in terms of isolates but that there is correlation between biofilm formation and resistance to antibiotics, with the strong biofilm producers resistant to more than one antibiotic.

Conclusion:

This study is the first in Algeria to describe microbiological characteristics of ICD infections. CoNS were the most frequent cause of ICD infections in our study but *Pseudomonas aeruginosa* and *Serratia* spp were also significant cause of bacteremia in patients with ICD, with comparable rate to the CoNS. This situation may imply bacteria contamination or poor technique during surgical procedure for implantation of the ICDs.

The bacteria in the biofilm were protected, more resistant and tolerated high concentrations of antibiotics, and thus played a major role in the development of ICD infections. Despite the improvements in ICD design and implantation techniques, ICD infection remains a serious challenge. It is therefore essential that proper infection prevention and control practices be put in place as well as strict indications for antibiotic prophylaxis during implantation of ICDs.

Conflict of interest statement:

No conflict of interest is declared.

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**Original Article****Open Access**

Effects of certain disinfectants and antibiotics on biofilm formation by *Staphylococcus aureus* isolated from medical devices at the University Hospital Center of Sidi Bel Abbès, Algeria

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Abstract:

Background: *Staphylococcus aureus* is one of the species of bacteria most frequently isolated from medical devices. The ability to produce biofilm is an important step in the pathogenesis of these staphylococci infection, and biofilm formation is strongly dependent on environmental conditions as well as antibiotics and disinfectants used in the treatment and prevention of infections.

Methodology: In this study, 28 *S. aureus* isolated from medical devices at the University Hospital Center of Sidi Bel Abbès in Northwestern Algeria were tested for biofilm formation by culture on Red Congo Agar (RCA). The tube method (TM) and tissue culture plate (TCP) techniques were also used to investigate the effect of penicillin, ethanol and betadine on pre-formed biofilm.

Results: Nineteen *S. aureus* isolates produced biofilm on the RCA and 7 produced biofilms by the tube method, 2 of which were high producer. In addition, 9 *S. aureus* isolates produced biofilm on polystyrene micro-plates, and in the presence of penicillin and ethanol, this number increased to 19 and 11 biofilm producing *S. aureus* isolates respectively. On the other hand, no biofilm was formed in the presence of betadine.

Conclusion: It is important to test for biofilm formation following an imposed external constraint such as disinfectants and antibiotics in order to develop new strategies to combat bacterial biofilms but also to better control their formation.

Keywords : *Staphylococcus aureus*, biofilm, medical device, disinfectant, antibiotic

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Effets de certains désinfectants et antibiotiques sur la formation de biofilms par *Staphylococcus aureus* isolé à partir de dispositifs médicaux au Centre Hospitalier Universitaire de Sidi Bel Abbès, Algérie

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Abstrait:

Contexte: *Staphylococcus aureus* est l'une des espèces de bactéries les plus fréquemment isolées des dispositifs médicaux. La capacité de produire du biofilm est une étape importante dans la pathogenèse de ces infections à staphylocoques, et la formation de biofilm dépend fortement des conditions environnementales ainsi que des antibiotiques et des désinfectants utilisés dans le traitement et la prévention des infections.

Méthodologie: Dans cette étude, 28 *S. aureus* isolés à partir de dispositifs médicaux au Centre hospitalier universitaire de Sidi Bel Abbès dans le nord-ouest de l'Algérie ont été testés pour la formation de biofilm par culture sur gélose rouge du Congo (RCA). La méthode des tubes (TM) et les techniques de plaques de culture tissulaire (TCP) ont également été utilisées pour étudier l'effet de la pénicilline, de l'éthanol et de la bétadine sur le biofilm préformé.

Résultats: Dix-neuf isolats de *S. aureus* ont produit un biofilm sur le RCA et 7 ont produit des biofilms par la méthode des tubes, dont 2 étaient très productifs. De plus, 9 isolats de *S. aureus* ont produit du biofilm sur des microplaques en polystyrène, et en présence de pénicilline et d'éthanol, ce nombre est passé à 19 et 11 isolats de *S. aureus* producteurs de biofilm respectivement. En revanche, aucun biofilm ne s'est formé en présence de bétadine.

Conclusion: Il est important de tester la formation de biofilm suite à une contrainte externe imposée comme les désinfectants et les antibiotiques afin de développer de nouvelles stratégies pour lutter contre les biofilms bactériens mais aussi pour mieux contrôler leur formation.

Mots-clés: *Staphylococcus aureus*, biofilm, dispositif médical, désinfectant, antibiotique

Introduction:

Staphylococcus aureus is a major cause of nosocomial and community-acquired infections. This organism is responsible for acute and chronic infections, most of which are due to the ability of the organism to adhere to medical implants and form biofilm (1). Biofilm is recognized as the most predominant form of bacteria development in nature, and is made of complex communities of microorganisms embedded in a self-secreted matrix of extracellular polymeric substances (EPS) (2).

Biofilms form on the surface of most materials either biological or non-biological. According to the Center for Disease Control and Prevention (CDC), 65% of bacterial infections are due to the presence of biofilms. In addition, infections associated with biofilms constitute a major clinical problem and are the cause of increased mortality, and generally associated with higher costs of medical treatment (3-5). The development of biofilms depends strongly on the environmental conditions in which they are formed, and the different parameters these biofilms are subjected to. Any change in these parameters is usually perceived as stress and can trigger a particular response within the biofilm (6).

It is therefore essential to study the response of biofilms to an imposed external constraint such as disinfectants and antibiotics, in order to develop adequate strategies to combat biofilms and also control and eventually limit their formation. The objectives of this study are to evaluate the capacity of *S. aureus* isolated from medical devices at the University Hospital of Side Bel Abbès, Algeria to adhere to surfaces and form biofilm, and to investigate the influence of

some disinfectants and antibiotics on the formation of biofilms.

Materials and methods:

Study setting and bacterial isolates

The *S. aureus* strains used in this study were isolated from medical devices at the Departments of Reanimation, Urology and Internal Medicine of the University Hospital Center (CHU), Sidi Bel Abbes, a city located in northwestern Algeria.

Isolation/identification of *S. aureus* isolates

After ablation of the medical devices, the microbiological analysis was carried out using the "Brun-Buisson" technique (7) which consists of rinsing the catheter lumen with saline solution and vortexing this content out through its intravascular end for culture on Chapman agar medium for selective growth isolation of staphylococci. Identification of *S. aureus* was done by conventional methods including colony morphology, Gram stain reaction, catalase production, and coagulase assay, and by the API STAPH system (Bio Mérieux®, France).

Detection of biofilm formation by Red Congo Agar (RCA) method

The Congo Red test was performed as previously described by Freeman et al., (8), which is based on the ability of the Congo Red dye to directly interact with certain polysaccharides, forming colored complexes (9). The medium consisted of Brain Heart infusion broth (BHIB, 37g/L), sucrose (50g/L), agar no. 1 (10g/L) and Congo Red stain (0.8g/L). The freshly prepared Congo Red agar plates were inoculated and incubated aerobically for 24 to 48 h at 37°C.

Biofilm producers form black colonies on CRA, while non-producers formed red colonies.

Detection of biofilm formation by tube method

This technique, developed in 1982 by Christensen et al., (11) provides a qualitative assessment of the biofilm formation. From a young culture of 24h, a colony is grown in 10mL brain heart infusion broth (BHIB) supplemented with 2% sucrose. After incubation at 37°C for 24hours, the tubes were washed with phosphate buffered saline (PBS) at pH7.3, and then dried. Each tube was then stained with crystal violet (0.1%) for 5 minutes. Once the dye is removed, the tubes were washed with distilled water and allowed to dry. Biofilm is considered formed when a visible film doubles the wall of the tube as well as its bottom. The formation of a ring at the liquid interface is not indicative of biofilm formation (11).

Detection of biofilm formation by Tissue Culture Plate (TCP) method

Quantitative determination of biofilm formation in 96-well microplates was performed according to Christensen et al., (10) with slight modification by extending the incubation time to 48 hours. After culturing the bacterial strains in the BHIB medium and incubating for 18h at 37°C, the mixture was diluted 1/100 in fresh BHIB medium. The wells of a 96 microtiter plates were then filled with 0.2ml of this dilution and incubated at 37°C. The microplate wells were washed 3 times with distilled water, dried in an inverted position, and stained with 0.5% (p:v) crystal violet solution. The adherent cells were resuspended in 95% ethanol solution and the absorbance measured at 540nm using an ELISA autoreader (Model 680, Biorad, UK). The isolates were then classified into three categories as: (a) non adhering, with an optical density less than 0.120; (b) weakly adhering, with an optical density greater than 0.120 but less than or equal to 0.240 and (c) strongly adhering, with an optical density greater than 0.240.

Effects of antiseptics and antibiotics on biofilm formation using the TCP technique

The antiseptics tested in this study were the main ones used at the Hospital University Center of Sidi Bel Abbes, which are polyvidone iodine (PVPI), marketed as 10% Betadine® (Laprophan Laboratory) and 70% ethyl alcohol prepared at the laboratory of the hospital pharmacy of the University Hospital. The antibiotic tested was penicillin G (1 million unit) which is marketed by SAIDAL laboratories.

After forming a 48-hour young biofilm by the TCP technique (as previously described), the 96-well microplate was rinsed

3 times with distilled water and dried. Then, Penicillin G (1 million unit), betadine 10% (an iodinated derivative) and 70% ethyl alcohol were added to the biofilm. The microplate was incubated for 24 hours. After incubation, the wells of the microplate were carefully rinsed, dried and stained with crystal violet according to the standard technique. The optical density (OD) was measured at 490 nm by the ELISA autoreader.

Results:

Biofilm formation by the different methods

A total of 28 *S. aureus* isolates were identified by conventional biochemical test and the API 20 Staph identification. Nineteen of the 28 *S. aureus* isolates produced biofilm (slime) by the CRA method, showing black colonies with dry crystalline consistency from production of exopolysaccharide that reacted with the Congo Red dye. By the tube method, only 7 *S. aureus* isolates produced biofilm, of which 2 were high producers (Table 1). The quantitative determination of biofilm formation by the TCP using the BHIB growth medium (Fig 1) shows that only 9 *S. aureus* isolates produced biofilm, with 7 of them were low producers and 2 high producers (Table 1).

Effects of antiseptics and antibiotic on biofilm formation by TCP method

Eleven *S. aureus* isolates produced biofilm in the presence of ethanol (70% ethyl alcohol) with 3 high and 8 moderate biofilm producers while 19 *S. aureus* isolates produced biofilm in the presence of penicillin (1mu) with 8 high and 11 moderate biofilm producers (Table 1 and Fig 1). On the other hand, no *S. aureus* isolate formed biofilm in the presence of betadine.

Discussion:

Staphylococcus aureus is one of the most common microorganisms responsible for infections of foreign body such as central venous catheters, mechanical heart valves and urinary catheters. Their major virulence factors are the ability to produce an extracellular matrix and form biofilm, which makes clinical treatment extremely difficult (12). Early detection of staphylococcal biofilms may be one of the essential steps for the prevention and treatment of infections of medical devices (13).

The finding of this study revealed that 19 of the 28 (67.9%) *S. aureus* isolates produce biofilm (slime) by culture on Congo Red agar, which agrees with 60.8% reported by Arciola et al., (14). Biofilm (slime) production give the appearance of black colo-

Table 1: Results of biofilm formation by *Staphylococcus aureus* isolated from medical devices.

Isolate	Unit	Medical device	TCP				TM	Production of biofilm
			BHIB	Ethanol	Penicillin	Betadine		
S1	Urology	U.C	-	++	++	-	-	+
S2	Urology	U.C	-	-	++	-	-	+
S3	Urology	U.C	-	-	++	-	-	+
S4	Urology	U.C	+	+	++	-	+	+
S5	Urology	U.C	-	-	+	-	+	+
S6	Urology	U.C	-	-	-	-	-	+
S7	Urology	U.C	-	-	+	-	-	+
S8	Urology	U.C	-	-	-	-	-	-
S9	Urology	U.C	-	-	-	-	-	-
S10	Urology	U.C	-	-	-	-	-	-
S11	Urology	U.C	-	-	+	-	-	-
S12	Urology	U.C	+	+	++	-	+	+
S13	Urology	U.C	-	-	-	-	-	-
S14	Intensive care	C.V.C	-	-	+	-	-	-
S15	Intensive care	C.V.C	-	-	-	-	-	-
S16	Intensive care	C.V.C	-	-	-	-	-	-
S17	Intensive care	C.V.C	-	-	+	-	-	+
S18	Intensive care	C.V.C	-	+	++	-	+	+
S19	Intensive care	C.V.C	-	+	++	-	+	+
S20	Intensive care	C.V.C	-	-	-	-	-	+
S21	Intensive care	C.V.C	-	+	+	-	-	+
S22	Intensive care	C.V.C	++	++	+	-	++	+
S23	Internal Medicine	U.C	+	+	+	-	-	+
S24	Internal Medicine	U.C	+	+	++	-	-	+
S25	Internal Medicine	U.C	+	+	+	-	-	+
S26	Internal Medicine	C.V.C	-	-	-	-	-	+
S27	Internal Medicine	C.V.C	-	-	+	-	-	+
S28	Internal Medicine	C.V.C	++	++	+	-	++	+

C.V.C: central venous catheters, U.C: urinary catheters, (++): biofilm formation good, (+): biofilm formation average; (-): non biofilm formation

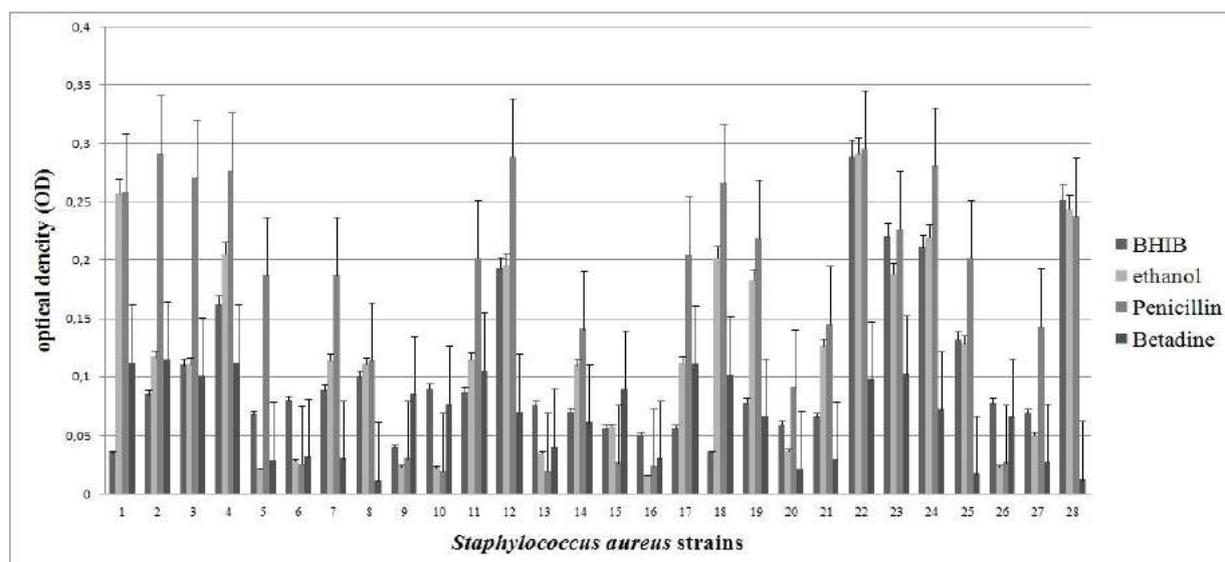


Fig 1: Biofilm formation of *Staphylococcus aureus* strains on BHIB, ethanol 70%, Penicillin and betadine 10%. Adherent bacterial biofilms were stained with Crystal violet as described in Materials and methods. A strain was considered biofilm-positive, if its OD was higher or equal to 0.120; $p < 0:05$ (t-test). Data are representative of 3 replicate experiments.

nies on Congo Red agar and is mainly due to the production of polysaccharide intercellular adhesin (PIA) that reacts with the culture

medium. Described for the first time in *Staphylococcus epidermidis* by Mack et al., (15), the PIA-encoded by *ica* locus, is

generally located on the surface of the cell, and plays an important role in intercellular adhesion. PIA has been recognized as a crucial factor in the colonization of medical equipment by staphylococci (12, 13, 16).

The result obtained by the TCP technique revealed that only 9 of the 28 (32.1%) *S. aureus* isolates produced biofilm in the BHIB medium. These results are in agreement with the observations of other authors who have shown that few strains are biofilm-forming in a growth medium without supplement such as (sugar, antibiotics or NaCl (11,17,18). In this study, 7 of the 28 (25%) *S. aureus* isolates produced biofilm by the tube method (TM) and a good correlation was noted between the TCP and TM for high biofilm producing isolates. However, there was a wide variability in biofilm classification by the TM method and difficulty in differentiating between low biofilm producing and non-producing isolates. Therefore, the present study agrees with the findings of other authors including Mathur et al., (11), TM method for routine detection of biofilm is not recommended. Consequently, the TCP technique is considered the 'gold standard' test for the detection of biofilm formation, and has been recognized as the most sensitive, accurate and reproducible method for detection of staphylococcal biofilm formation. It also allows for quantitative assessment in order to compare the adhesion of different strains and to examine large number of isolates simultaneously (19), as well as allows for the identification of optimal culture factors and conditions for biofilm formation (20).

Biofilm formation is a complex phenomenon, which can be affected by many factors, particularly the surrounding environment (21). In order to study the influence of certain factors in the surrounding environment at the Hospital University Center of Sidi Bel Abbes on biofilm formation in *S. aureus*, the TCP technique was selected for testing the effects of two commonly used antiseptics (ethanol and betadine) and penicillin G. We observed that after addition of penicillin and ethanol, the number of biofilm-forming *S. aureus* isolates increased to 19 and 11 respectively. These observations are consistent with those from other studies (11,22), which suggest a strong dependence between growth conditions and biofilm formation in staphylococci. Luther et al., (23) and Redelman et al., (24) observed in their studies that ethanol encouraged biofilm formation in all strains studied. Similarly, El-Banna et al., (25) observed that antibiotics promote biofilm formation in staphylococci isolated from medical devices at University Hospital Center of Alexandria City in Egypt.

Based on our 2013 results (2) and

those of others in the literature, it has been found that the expression of *ica* operon depends on environmental conditions such as growth media composition, temperature, osmolarity, the presence of oxygen and sub-inhibitory concentrations of antibiotics. High concentrations of NaCl also increase biofilm formation by strongly inducing *ica* operon expression in staphylococci. The presence of divalent cations such as calcium and magnesium, increases the production of polysaccharides, which leads to amplification of biofilm formation. In staphylococci, the expression of the *ica* ADBC genes can also be influenced by other environmental conditions such as oleic acid and iron limitation (12,26,27).

On the other hand, the resistance of biofilm-forming bacteria to antibiotics and disinfectants is well acknowledged. According to some authors, resistance is attributed to factors such as bacteria physiology, power of matrices, and other factors (28). Repeated exposure to disinfectants and antibiotics can generate some physiological adaptations that further delay the subsequent tolerance of the biofilm. When a community of adherent bacterial cells was subjected to antibiotics and disinfectants, only a few were able to resist them (33). On the other hand, in the presence of betadine (polyvidone iodine), the optical density decreases in all the isolates and none of them was able to form a biofilm.

The results of Essayagh et al., (34) agrees with ours that polyvidone iodine (PVPI) is the best of antiseptics studied. In fact, only 6 (4.6%) out of the 130 strains tested in their study could resist PVPI that was available at the pharmacy while 40 (30.7%) were resistant to iodinated alcohol and 20 (15.4%) to 70% alcohol. Chemical analysis has previously confirmed this finding (35). Indeed, PVPI is a stable molecule consisting of an iodine complex and a water-soluble organic agent that slowly transports and releases iodine. This structure makes the PVPI less irritating and allergenic, and more stable over time while iodized alcohol and 70% ethyl alcohol are stable only over fifteen days and one month respectively after the date of their preparations (35, 36).

Conclusion:

S. aureus isolates exposed to clinically relevant concentrations of ethanol increase biofilm formation, however, no strain formed biofilm in the presence of betadine. Future research should determine the impact of our findings on various alcohol preparations used in the management and prevention of clinical infections caused by biofilm forming staphylococci.

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**Original Article****Open Access**

Nasal carriage of methicillin resistant *Staphylococcus aureus* among medical students of a private institution in Ilishan-Remo, Ogun State, Nigeria

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Abstract:

Background: Nasal carriage of methicillin resistant *Staphylococcus aureus* (MRSA) is a major factor for its transmission especially from the health workers and medical students to their patients. There are a number of published data on the prevalence of MRSA among health workers but data on nasal colonization of medical students by MRSA are sparse in Nigeria. The objectives of this study are to determine the prevalence of nasal carriage of MRSA among medical students of the Ben Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria, and identify risk factors associated with this nasal carriage.

Methodology: A case control study involving 100 clinical (study group) and 100 pre-clinical (control group) medical students was undertaken between March 2018 and October 2019. Structured questionnaire was administered to obtain socio-demographic information and potential risk factors. Nasal swab was collected from each student and cultured for isolation of *S. aureus* by standard microbiology techniques. Phenotypic MRSA was detected by the cefoxitin 30µg disk diffusion method according to the guideline of Clinical and Laboratory Standards Institute. The *mecA* gene was detected by conventional polymerase chain reaction (PCR) assay.

Results: The prevalence of *S. aureus* nasal carriage among the study group was 14% (14/100) while the prevalence among the control group was 6% (6/100) ($p=0.097$). The prevalence of phenotypic MRSA among the study group was 4% (4/100) and 1% (1/100) among the control group ($p=0.3687$) while *mecA* gene was detected in 3 of the 4 (75%) phenotypic MRSA positive study participants and in the only (100%) phenotypic MRSA positive (1%) control group. Antibiotics usage without prescription, antibiotic treatment of common cold, and use of antibiotics in the previous one year, were significantly associated with MRSA carriage among the study group.

Conclusion: Although the prevalence of nasal carriage of *S. aureus* and MRSA among clinical and pre-clinical medical students was not statistically significant, the risk factors identified with carriage of MRSA among the study group indicates the need for antimicrobial stewardship program to reduce carriage and transmission of MRSA by medical students.

Keywords: methicillin resistant, *Staphylococcus aureus*, *mecA* gene, nasal carriage, medical students

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Transport nasal de *Staphylococcus aureus* résistant à la méthicilline parmi les étudiants en médecine d'un établissement privé, Ilishan-Remo, État d'Ogun, Nigéria

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Abstrait:

Contexte: Le portage nasal de *Staphylococcus aureus* résistant à la méthicilline (SARM) est un facteur majeur pour sa transmission, en particulier des agents de santé et des étudiants en médecine à leurs patients. Il existe un certain nombre de données publiées sur la prévalence du SARM parmi les agents de santé, mais les données sur la colonisation nasale des étudiants en médecine par le SARM sont rares au Nigéria. Les objectifs de cette étude sont de déterminer la prévalence du portage nasal de SARM chez les étudiants en médecine de la Ben Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria, et d'identifier les facteurs de risque associés à ce portage nasal.

Méthodologie: Une étude cas-témoins portant sur 100 étudiants en médecine cliniques (groupe d'étude) et 100 précliniques (groupe témoin) a été entreprise entre mars 2018 et octobre 2019. Un questionnaire structuré a été administré pour obtenir des informations sociodémographiques et des facteurs de risque potentiels. Un écouvillon nasal a été prélevé sur chaque élève et cultivé pour l'isolement de *S. aureus* par des techniques de microbiologie standard. Le SARM phénotypique a été détecté par la méthode de diffusion sur disque de céfoxitine 30µg conformément aux directives de la Institut des normes cliniques et de laboratoire. Le gène *mecA* a été détecté par un essai classique de réaction en chaîne par polymérase (PCR).

Résultats: La prévalence du portage nasal de *S. aureus* dans le groupe d'étude était de 14% (14/100) tandis que la prévalence dans le groupe témoin était de 6% (6/100) ($p=0,097$). La prévalence du SARM phénotypique parmi les groupe d'étude était de 4% (4/100) et 1% (1/100) dans le groupe témoin ($p=0,3668$) tandis que le gène *mecA* a été détecté chez 3 des 4 (75%) participants phénotypiques MRSA positifs à l'étude et dans le seul (100%) groupe témoin phénotypique SARM positif (1%). L'utilisation d'antibiotiques sans ordonnance, le traitement antibiotique du rhume et l'utilisation d'antibiotiques au cours de l'année précédente étaient significativement associés au portage du SARM dans le groupe d'étude.

Conclusion: Bien que la prévalence du portage nasal de *S. aureus* et du SARM chez les étudiants en médecine clinique et préclinique n'était pas statistiquement significative, les facteurs de risque identifiés avec le portage du SARM dans le groupe d'étude indiquent la nécessité d'un programme d'intendance antimicrobienne pour réduire le portage et transmission du SARM par les étudiants en médecine.

Mots-clés: résistant à la méthicilline, *Staphylococcus aureus*, gène *mecA*, portage nasal, étudiants en médecine

Introduction:

Staphylococcus aureus is one of the most common bacterial causes of infection in the community and healthcare settings (1). However, the emergence of the drug resistant strains of *S. aureus* especially, methicillin resistant *S. aureus* (MRSA), has become a global threat (2,3) with reports from many United State hospitals and communities (4). According to a report on antimicrobial resistance by the World Health Organization (WHO), patients with MRSA infections are estimated to be 64% more likely to die than people infected with the non-resistant *S. aureus* strain (5).

In addition, patients with MRSA infections have twice as much hospital bills paid than those who are infected with methicillin sensitive *S. aureus* (MSSA) due to longer duration of illness, additional tests and use of more expensive drugs (3). In Nigeria, MRSA prevalence of

28.6% to 81% has been reported in different parts of the country among hospital in-patients (2,6,7). Methicillin resistance in *S. aureus* is due to acquisition of *mecA* gene that encodes an abnormal penicillin-binding protein 2a (PBP2a), and recently a similar gene called *mecC* gene has been described as a cause of MRSA (8).

The anterior nares are the main reservoirs of MRSA, although other body sites are frequently colonized such as the hands, skin, axillae, and intestinal tract (9,10). Nasal carriage of MRSA is a major factor for transmission of this pathogen (9). Healthcare workers (HCWs) are important in the transmission of MRSA because they are at higher risk of colonization than the general public, apparently due to increased exposure to this organism (2,11,12). Transmission occurs during contact with patients when infection control measures are not adhered to (12).

Aside HCWs, medical students can be

potential nasal carriers of MRSA and can aid in transmission of this pathogen within hospitals because of exposure to patients and other healthcare workers during clinical rotations (13). In Louisiana, exposure of medical students to the hospital environment was reported to have increased the prevalence of MRSA nasal colonization from 0% to 3.2% (14). In Taiwan, the MRSA carriage rate amongst medical students was reported to be 2.2% (15) while in Saudi Arabia, the prevalence rate was 6.7% (13). It has been reported that the prevalence of MRSA in health institutes is directly proportional to the morbidity and mortality caused by the strains (16). Therefore, screening for MRSA in hospitals is an important factor for building up successful infection control strategies.

In Nigeria, data on MRSA nasal colonization of medical students are sparse. Therefore, the objectives of this study are to determine the prevalence of MRSA nasal carriage rate among medical students of the Ben Carson School of Medicine, Ilishan-Remo, Ogun State, Nigeria, and identify risk factors associated with MRSA nasal carriage among the study participants.

Materials and method:

Study setting, design, subjects and sampling method

This was a case control study in which 100 clinical (study group) and 100 pre-clinical (control group) medical students of the Ben Carson School of Medicine, Babcock University were recruited for the study. The sample size of 100 was calculated for the study based on 6.7% prevalence of MRSA among medical students in Saudi Arabia to give a 95% confidence level and margin of error of 5%.

The controls were matched and recruited in ratio 1:1 with the study group. A simple random sampling using ballot without replacement technique was used to recruit the participants for the study. The study was conducted between March 2018 and October 2019.

Ethical approval

Ethical approval was obtained from Babcock University Ethical review committee before commencement of the study. Informed consent of each participant was also obtained.

Data and sample collection

A semi structured, pre-tested questionnaire was interviewer-administered to each student participant to obtain socio-demographic information and attributes considered risk factors for MRSA nasal colonization. Nasal swab

was collected from each participant using moist sterile cotton tip swab and transported to the laboratory for analysis.

Isolation and identification of *Staphylococcus aureus*

The nasal swabs were inoculated onto Blood agar plates and incubated aerobically at 37°C for 24 hours. Colonies on culture plates were identified and confirmed as *S. aureus* by Gram stain reaction, catalase test, tube coagulase test and growth on mannitol-salt agar (a selective medium for *S. aureus*) (17). Antibiotic susceptibility test on each *S. aureus* isolate was done by the modified Kirby Bauer disk diffusion method (18).

Phenotypic methicillin resistance detection

All identified *S. aureus* isolates were screened for methicillin resistance by the cefoxitin disk diffusion test using 30µg disk on Mueller Hinton agar. Inhibition zone diameter of ≤21 mm was reported as oxacillin (methicillin)-resistant and >21 mm as oxacillin (methicillin)-sensitive (19). Methicillin sensitive *S. aureus* (MSSA) ATCC 25923 was used as negative control and MRSA ATCC 43300 served as positive control strain (19).

Detection of *mecA* gene by PCR assay

DNA extraction was done using DNA extraction kit (Zymo Research Quick-DNA Fungal/ Bacteria Miniprep Kit) following the manufacturer's instruction. The *mecA* gene was amplified using previously described primers *mecA*-F-5'-GTGGAATTGGCCAATACAGGAAC-3' and *mecA*-R-5'-GTTAGTTGAATATCTTTGCCATC-3' (Inqaba) which produced a 502 bp amplicon (13). The 25µl PCR volume consist of 12.4µl nuclease free water, 2.5µl of 10xPCR buffer, 2µl of 25mM MgCl₂, 1µl each of forward and reverse primers, 1µl of DMSO, 2µl of 2.5mM dNTPs, 0.1µl of 5µ/µl Taq DNA polymerase and 3µl of extracted template DNA.

The reaction was amplified in a PCR thermal cycler (Applied Biosystems Gene Amp PCR system 9700) of 9 cycles of initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 15 seconds, annealing temperature of 65°C for 20 seconds, extension at 72°C for 30 seconds and holding temperature at 10°C. This was followed by 35 cycles of denaturation at 94°C for 15 seconds, annealing temperature of 55°C for 20 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 7 minutes. The PCR products were run on 2.5% agarose gel with ethidium bromide dye and visualized under ultraviolet transilluminator and photographed. Amplicon size of 502 bp was considered positive for *mecA* gene (13).

Results:

A total of 200 medical students were studied; 100 in the clinical (study group) and 100 in the pre-clinical (control group) rotation. The socio-demographic characteristics of the participants as depicted in Table 1 showed that the two groups were well matched in gender and other characteristics. Expectedly however, majority of the participants in the study group (67%) were aged 21-25 years while majority of participants in the control group (92%) were aged 15-20 years.

The prevalence rate of *S. aureus* nasal carriage among the study group was 14% (14/100) while the rate was 6% (6/100) among

the control group ($p=0.0970$). Similarly, the prevalence rate of MRSA nasal carriage among the study group was 4% (4/100) while the rate was 1% (1/100) among the control group ($p=0.3687$) (Table 2). There was no statistically significant relationship between the prevalence rate of *S. aureus* and MRSA nasal carriage in the study and that of the control group. The prevalence rate of methicillin resistance in the *S. aureus* population from the study group was 28.6% (4/14) while the rate in the control group was 16.7% (1/6). Out of the 4 phenotypic MRSA isolates from the study group, 3 (75%) were *mecA* positive while the only MRSA isolate (100%) from the control group was *mecA* positive ($p=0.312$) (Fig 1).

Table 1: Socio-demographic characteristics of the clinical (study group) and pre-clinical (control) medical students of Ben Carson School of Medicine, Ilishan-Remo, Ogun State, Nigeria

Socio-demographic variables	Study group No (%)	Control group No (%)
Age group (years)		
15-20	32 (32)	92 (92)
21-25	67 (67)	7 (7)
26-30	1 (1)	1 (1)
Gender		
Male	40 (40)	44 (44)
Female	60 (60)	56 (56)
Marital status		
Single	94 (94)	97 (97)
Married	6 (6)	3 (3)
Family type		
Monogamous	97 (97)	94 (94)
Polygamous	3 (3)	6 (6)
Religion		
Christianity	93 (93)	95 (95)
Islam	7 (7)	5 (5)

Table 2: Comparisons of the prevalence of nasal carriage of *Staphylococcus aureus* and MRSA between clinical and pre-clinical medical students of Ben Carson School of Medicine, Ilishan-Remo, Ogun State, Nigeria

Variable	Groups		Fisher Exact Statistics	
	Study (%)	Control (%)	OR	p value
<i>Staphylococcus aureus</i>			2.550	0.0970
Positive	14 (14)	6 (6)		
Negative	86 (86)	94 (94)		
MRSA			4.125	0.3687
Positive	4 (4)	1 (1)		
Negative	96 (96)	99 (99)		

MRSA=methicillin resistant *Staphylococcus aureus*; OR = Odds Ratio

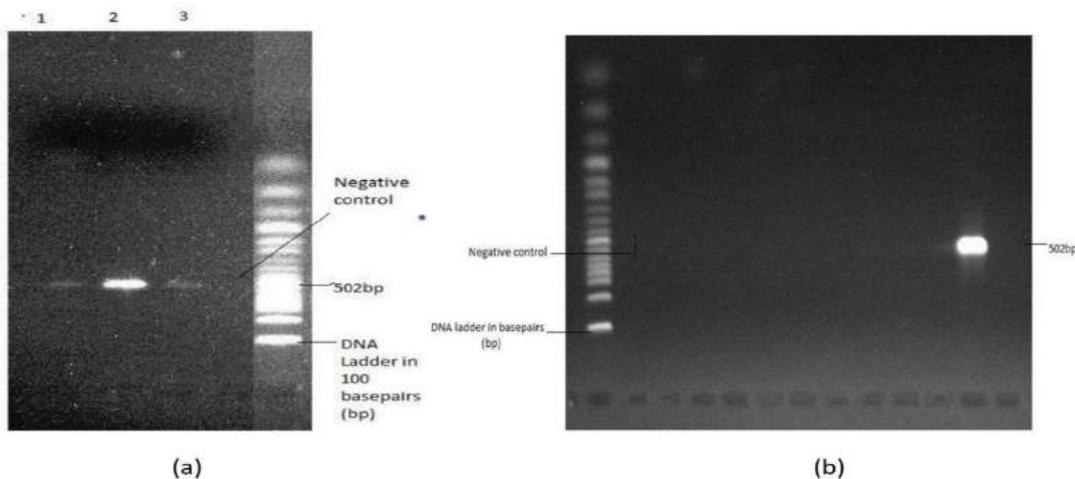


Fig 1: Gel electrophoresis picture of the amplified *mecA* gene of the 3 *S. aureus* isolates in the study group (a) and one *S. aureus* isolate in the control group (b)

Table 3: Factors associated with MRSA nasal carriage among clinical medical students of Ben Carson School of Medicine, Ilishan-Remo, Ogun State, Nigeria

Factors	Sub-variables	MRSA (n=100)		Statistics	
		Yes (%)	No (%)	X ²	p-value
Age in years	15-25	3 (2.4)	29 (90.6)	3.546	0.1698
	26-30	1 (1.5)	66 (98.5)		
	31-35	0	1 (100)		
				OR	p value
Gender	Male	2 (5)	38 (95)	1.526	1.0000
	Female	2 (3.3)	58 (96.7)		
Marital status	Single	4 (4.3)	90 (95.7)	0.6464	1.0000
	Married	0	6 (100)		
Family type	Monogamous	4 (4.3)	90 (95.7)	0.6464	1.0000
	Polygamous	0	6 (100)		
Religion	Christianity	3 (3.2)	90 (96.8)	0.2000	0.2554
	Islam	1 (14.3)	6 (85.7)		
Antibiotics in the last three months	Yes	1 (20)	4 (80.0)	7.667	0.1881
	No	3 (3.2)	92 (96.8)		
Antibiotics without prescription	Yes	3 (25)	9 (75)	29.00	0.0051*
	No	1(1.1)	87 (98.9)		
Recent hospital admission	Yes	0	2 (100)	4.200	1.0000
	No	4 (4.1)	94 (95.9)		
Antibiotics for common cold	Yes	3 (60)	2 (40)	141.00	0.0002*
	No	1 (1.05)	94 (98.05)		
Recent surgery	Yes	0	8 (100)	1.157	1.0000
	No	4 (4.3)	88 (95.7)		
Use antibiotics in the last one year	Yes	3 (15)	17 (85)	13.942	0.0245*
	No	1 (1.25)	79 (98.75)		

X²=Chi square; OR = Odds Ratio; *p value less than 0.05 is considered significant

Antibiotic usage without prescription, usage of antibiotics to treat common cold and use of antibiotics in the last one year were all significantly associated with MRSA nasal carriage among the study group (Table 3).

Discussion:

MRSA cause infections in both the hospital and community, and healthcare workers and medical students can be the route

of infection especially when infection control practices are not adhered to (13). In this present study, the prevalence of MRSA nasal carriage among clinical medical student was 4%. In Nigeria, data on the prevalence of MRSA nasal carriage among the clinical students are sparse, however in other parts of the world, our finding is similar to that of Baliga et al., (20) in Turkey who reported a prevalence of 4.4% among medical students on clinical rotation and Piechowicz et al., who reported a prevalence of 4.5% (21). Other studies across the world have reported different prevalence rates among clinical students; Bettin et al., (14) in Columbia reported 1.6%, Bellows et al., (15) reported 3.2% in New Orleans, Chen et al., (22) reported 1.9% in Taiwan, and Jujena et al., (23) reported a prevalence of 9% in India. The different rates reported from different parts of the world might be related to the level and degree of adherence to standard infection control practices in hospitals where medical students undergo their clinical rotations.

In this present study, the prevalence of 4% for MRSA nasal carriage among the clinical students was higher than the 1% among the pre-clinical students, and similarly with *S. aureus* nasal carriage (14% versus 6%) but the differences in rates were not statistically significant, probably due to small number of cases. However, frequent exposure to the hospital is known to play a role in nasal colonization by MRSA (13), which could be responsible for the higher colonization, albeit statistically insignificant, of the clinical students who are more exposed to hospital at this stage of their training.

Other studies have reported higher colonization rate in clinical than pre-clinical students, Bellows et al., (14) reported that exposure of students to hospital clinical rotation increased the prevalence of MRSA nasal carriage from 0 to 3.2%, and Slifka et al., (24) in the USA reported the prevalence of MRSA among students with and without significant healthcare associated exposure to be 3.4% versus 2.1%. Also, Peichowicz et al., (21) in their study reported that 21% of clinical students were colonized by MRSA while all preclinical students were negative. In Saudi Arabia, Zakai et al., (13) reported that medical interns carry MRSA more than 6th year clinical students and students who were not exposed to clinical work. Although some other studies reported contrary results in which pre-clinical students had more nasal

colonization than clinical students (14,15), other risk factors responsible for nasal colonization such as antibiotic mis-use might be responsible for these contrary reports. All the same, studies conducted among students un-exposed to clinical duties in Thailand and Hungary have reported low prevalence rates of MRSA (25,26). Therefore, considering majority of the data, frequent hospital exposure among medical students might increase the prevalence of MRSA nasal colonization and education on standard infection control practices will help to reduce colonization among these students than the recommended mupirocin for decolonization because of some reported drawbacks of the decolonizer (27).

The *mecA* gene mediates methicillin resistance in *S. aureus* but one phenotypic MRSA isolate from the study group did not carry *mecA* gene, which may indicate that other *mec* genes aside *mecA* may be responsible for resistance in this isolate (8). Antibiotic usage without prescription, usage of antibiotics to treat common cold and use of antibiotics in the last one year were all significantly associated with MRSA nasal colonization among the study group. This finding is similar to previous studies in which recent use of antibiotics and use of antibiotics in the last six months were significantly associated to MRSA (15,23). These data showed that mis-use of antibiotics is a factor to consider in MRSA nasal colonization.

A major limitation in our study is the non-longitudinal nature of the design in which only one nasal swab sample was collected from the participants hence the new nomenclature of MRSA nasal carriage of persistent carriers and others (28), as distinct from the old nomenclature of persistent, intermittent and non-carrier, cannot be fulfilled with only one simple swab test.

Conclusion:

In conclusion, although the difference between the prevalence of nasal carriage of *S. aureus* and MRSA did not reach a statistically significant level, the prevalence rates were higher among the clinical than the pre-clinical students. Antibiotic usage without prescription, usage of antibiotics to treat common cold, and usage of antibiotics in the last one year, were all significantly associated with MRSA nasal colonization among the clinical (study group) students.

With these findings, awareness should

be raised among medical students of the need to adhere to standard infection prevention and control (IPC) practices to limit the spread of MRSA in the hospital. Emphasis on antimicrobial stewardship is pivotal to preventing emergence of antimicrobial resistant organisms such as MRSA.

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**Original Article****Open Access****Effects of highly active antiretroviral treatment on liver and renal functions of HIV-infected patients attending the day care clinic of the Bamenda Regional Hospital, Cameroon***¹Samje, M., ²Youego, E. K. J., ²Kefeyin, T. W., and ³Lukong, H.¹Department of Biomedical Sciences, Faculty of Health Sciences, University of Bamenda, P. O. Box 39, Bambili, Republic of Cameroon²Department Medical Laboratory Sciences, University of Bamenda, Cameroon³Department of Medical Laboratory Sciences, Kesmonds International University, Cameroon*Correspondence to: msamje@gmail.com; +237677679112**Abstract:**

Background: Though the development of antiretroviral therapy has brought some relief to the menace of HIV infections, the side effects and toxicity of these drugs can still present a major challenge to users, thus leading to a switch or discontinuation of treatment. The aim of this study was to determine the effect of antiretroviral treatment on some biochemical markers of liver and renal functions among HIV-infected patients receiving treatment at the Day Care Clinic of the Regional Hospital, Bamenda, Cameroon.

Methodology: A case control study design comprising 100 HIV-infected patients on highly active antiretroviral therapy (HAART) and 100 HIV-negative controls was conducted from February to May 2019. The serum activity of aspartate amino transferase (AST) and alanine amino transferase (ALT), and serum creatinine levels for each group were measured using BIOSMART 240 autoanalyzer. The estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft-Gault formula while proteinuria was determined with the CYBOW 11M strips. Data were analyzed using IBM SPSS version 21.0. Differences in the serum activity and levels of the biomarkers between the case and control groups were analyzed using the Chi-square test.

Results: The prevalence of transaminitis (AST and ALT >40.0 U/L) among HAART-treated participants was 26.0% for AST and 20% for ALT while that of HIV-negative controls were 15% and 12% respectively but the difference was not statistically significant ($p > 0.05$). Elevated serum creatinine level (> 1.4 mg/dL) was seen in 25% of HAART-treated group compared to 7% in HIV-negative control ($p = 0.0010$) while renal impairment ($eGFR < 60$ ml/min/1.73m²) was seen in 24% of HAART-treated and 14% of HIV-negative group ($p = 0.1048$). Elevated activity of transaminases and levels of creatinine, proteinuria and abnormal eGFR in HAART-treated patients were mostly seen in those who were on first line antiretroviral therapy, and those who have been on treatment for over 5 years.

Conclusion: HAART is associated with transaminitis and elevated serum creatinine but no increase in renal impairment compared to the general population. It is important that following initiation of HAART, liver and kidney functions are regularly monitored.

Keywords: HIV; HAART; AST; ALT; eGFR

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Effets du traitement antirétroviral fortement actif sur les fonctions hépatiques et rénales chez des patients infectés par le VIH assistant au centre de traitement de l'hôpital régional de Bamenda, Cameroun*¹Samje, M., ²Youego, E. K. J., ²Kefeyin, T. W., et ³Lukong, H.¹Département des sciences biomédicales, Faculté des sciences de la santé, Université de Bamenda, P. O. Box 39, Bambili, République du Cameroun

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Resumé:

Contexte: Bien que le développement de la thérapie antirétrovirale ait apporté un certain soulagement à la menace par l'infection au VIH, les effets secondaires et les toxicités de ces médicaments peuvent encore représenter un défi majeur pour les utilisateurs conduisant ainsi à un changement ou à l'arrêt du traitement. Le but de cette étude était de déterminer l'effet du traitement antirétroviral sur certains marqueurs biochimiques de la fonction hépatique et rénale chez les patients infectés par le VIH recevant un traitement au Centre de traitement antirétroviral de l'hôpital régional de Bamenda, au Cameroun.

Méthodologie: Un modèle d'étude cas-témoins comprenant 100 patients infectés par le VIH sous traitement antirétroviral hautement actif (TAHA) et 100 témoins séronégatifs a été mené de février à mai 2019. L'activité sérique de l'aspartate amino transférase (ASAT) et de l'alanine amino transférase (ALAT) et les niveaux de créatinine sérique pour chaque groupe ont été mesurés en utilisant l'autoanalyseur BIOSMART 240. Le débit de filtration glomérulaire estimé (DFGe) a été calculé en utilisant la formule Cockcroft-Gault tandis que la protéinurie a été déterminée avec les bandelettes CYBOW 11M. Les données ont été analysées à l'aide d'IBM SPSS version 21.0. Les différences dans l'activité sérique et les niveaux des biomarqueurs entre le groupe de cas et le groupe témoin ont été analysées à l'aide du test du chi carré.

Résultats: La prévalence de la transaminite (ASAT et ALAT > 40,0 U/L) chez les participants traités par TAHA était de 26,0% pour l'ASAT et 20% pour l'ALAT tandis que celle des témoins séronégatifs était respectivement de 15% et 12% mais la différence n'était pas statistiquement significative ($p > 0,05$). Un taux de créatinine sérique élevé (>1,4 mg/dL) a été observé chez 25% des patients traités par TAHA, contre 7% chez les témoins séronégatifs ($p = 0,0010$), tandis qu'une insuffisance rénale (DFGe <60 ml/min/1,73m²) était vu dans 24% des patients traités par TAHA et 14% des patients séronégatifs pour le VIH ($p = 0,1048$). Une activité élevée des transaminases et des niveaux de créatinine, de protéinurie et de DFGe anormal chez les patients traités par TAHA ont été principalement observés chez ceux qui étaient sous traitement antirétroviral de première ligne et ceux qui suivaient un traitement depuis plus de 5 ans.

Conclusion: la multithérapie est associée à une transaminite et à une élévation de la créatinine sérique mais pas d'augmentation de l'insuffisance rénale par rapport à la population générale. Il est important qu'après l'initiation de la multithérapie, les fonctions hépatiques et rénales soient régulièrement surveillées.

Mots-clés: VIH; TAHA; ASAT; ALAT; eGFR

Introduction:

Human immunodeficiency virus (HIV) leads to a depletion in CD₄⁺ cells thus fueling opportunistic infections and consequently reducing life expectancy (1). The HIV/AIDS pandemic is estimated to have killed more than 25 million people worldwide (2). The management of this disease normally includes the use of simple antiretroviral drugs. Several classes of antiretroviral agents acting on different stages of the HIV life cycle have been reported. Highly active antiretroviral therapy (HAART), multiple drugs with different viral targets have been shown to decrease total burden of HIV in infected patients and maintain the function of the immune system (3). The arrival of HAART has greatly enhanced long-term viral suppression, decrease opportunistic infections and increased quality of life (QoL) in HIV-infected population worldwide (4).

While the liver is known as the gate way of drugs to the body, the kidney serves as an exit of the metabolized substance from the body. The toxicity of HAART, especially on the liver and kidneys, has emerged as an important complication and eventually a major reason for antiretroviral therapy shift and/or discontinua-

tion of the offending drug (5). The use of antiretrovirals have been associated with liver disease. Although many sites in the body such as the gut, lungs and kidneys are involved in drug metabolism, the liver is the most metabolically active tissue and is known to be responsible for the majority of drug metabolism. It is hypothesized that following exposure to the drug, the toxic moiety induces some type of stress or functional disturbance, with mitochondrial injury being one of the most important targets recognized (6,7). The mechanisms for drug-induced liver injury also include dose-dependent toxicity, hypersensitivity reactions, and immune reconstitution (8).

The association between HIV infection and renal disease was first reported as far back as 1984 by researchers in New York and Miami (9,10). Renal disease in HIV can result from the use of HAART with their associated adverse effects (11) that may shorten the lifespan of patients. Several routes for drug elimination from the body exist with most drugs eliminated by pathways that involve kidneys or liver. Renal excretion plays an important role in eliminating unchanged drugs or their metabolites into urine. The diagnosis of HAART induced adverse effects on the kidney involves performing renal

function tests, a group of clinical biochemistry laboratory blood tests designed to give information about the state of a patients' kidney.

Considering the important role of the liver and kidney in respectively metabolizing or detoxifying drugs and excreting waste products of metabolism, these vital organs need to be monitored during therapies of different drug combinations and regimens in order to be sure that harm is not done to them. This study was therefore aimed at evaluating the effects of different HAART regimens on the liver and kidney functions of patients undergoing such treatment at the Bamenda Regional Hospital, Cameroon.

Materials and methods:

Study area

The study was carried out at the Day Care Clinic of the Bamenda Regional Hospital, Cameroon. The Bamenda Regional Hospital is in the metropolitan city of Bamenda with a population of over 650,000 inhabitants. It is the major Government hospital in the northwest region of Cameroon and serve as a referral hospital in this region.

Study design

A case-control study design was employed where the case group comprised HIV-infected subjects on HAART while the control group comprised HIV-negative individuals. The markers of liver and kidney functions were assessed and compared between the groups.

Subjects and selection criteria

The subjects were HIV-infected patients on HAART (case) and HIV negative individuals (control). The sample size for the study was determined using the Cochran's formula (12) with an estimated prevalence obtained from a study carried out by Wondifraw and others (13). The sample size of 200 was obtained; 100 subjects for the case group and 100 for the control group. Inclusion criteria for cases were; HIV-infection in patient within the age group 20-65 years on HAART with no underlying hypertension, diabetes, kidney diseases; not pregnant; not on any medication (except HAART); and negative for hepatitis B or C virus infection. The controls were apparently healthy subjects.

The Determine[®] HIV-1/2 Ag/Ab Combo (Alere Medical Co., Ltd, Matsuhidai, Matsudo-Shi, China) was used as first line HIV screening test while reactive samples were confirmed by the OraQuick (OraSure Technologies, Inc, Bethlehem) in line with national algorithm for HIV diagnosis in Cameroon.

Ethical approval

Ethical clearance for the study was obtained from the University of Bamenda Institutional Review Board while administrative authorization was obtained from the Director of the Hospital. Signed informed consent was equally obtained from the study subjects.

Data collection

A structured questionnaire was used to collect socio-demographic information. For patients who could not read or write, information was collected orally and then transcribed. Treatment details including types of drugs, duration of treatment together with other clinical parameters were obtained from the medical records of the subjects.

Specimen collection

From each subject participant, approximately 4 ml of venous blood was collected into a labelled dry specimen tube and 10 ml of urine into sterile urine sample bottle. The blood was allowed to clot and then centrifuged at 3000 rpm for 5 minutes to obtain serum for the measurement of serum activity of liver enzymes (AST and ALT) and serum creatinine level. Renal function was assessed by serum creatinine level and creatinine clearance by estimating the glomerular filtration rate (eGFR).

Measurement of ALT and AST activity

Serum activity of ALT and AST was measured using the BIOSMART 240 analyzer (BIONLINE-S.R.L). Samples were loaded in the tray of the analyzer and aliquot dispensed into the reaction vessel together with the reagents. After the solution was mixed, it passed through an in-built colorimeter which measured the absorbance and displayed the concentration of the analyte. Calibration was done before running each set of samples using reagents and quality control samples that was provided in the test kit (REF 92027 AST/GOT-ALT/GPT). The analyzer calculated and expressed ALT and AST activity in U/L in the reaction at 37°C within three minutes.

Interpretation of serum activity of the aminotransferases was based on the AIDS Clinical Trial Group grading system with ALT and AST elevations >40.0 U/L, which for adults, is the upper limit of normal (ULN) range in individuals with normal ALT and AST values at baseline. Hepatotoxicity grades were categorized as Grade 1 when ALT and AST values lie between 1.25 and <2.5 × ULN; Grade 2 when values lie between 2.5 and <5.0 × ULN; Grade 3 when values lie between 5.0 and <10 × ULN; and Grade 4 when values are ≥10 × ULN (14).

Measurement of creatinine

Serum creatinine level was measured automatically using the BIOSMART 240 analyzer as described for AST and ALT, except for the reagents and control samples. The results were compared with the normal range for creatinine (0.6-1.4 mg/dL).

Detection of urine protein

Protein was detected in urine qualitatively using the CYBOW 11M strips. Briefly, the tip of test strip was dipped into urine for a maximum of 5 seconds and deviation in normal color was evaluated visually with the aid of a color chart labelled on the strip container.

Creatinine clearance (Ccr) estimation

The estimated Glomerular Filtration Rate (eGFR) was calculated using the Cockcroft-Gault formula; $GFR = \frac{(140 - \text{age}) \times \text{weight}}{72 \text{Scr}}$ ($\times 0.85$ if female), which took into consideration serum creatinine (Scr), age, sex and weight of participant (15). The National Kidney Foundation clinical practice guideline was used to classify renal impairment based on the estimated GFR values. The eGFR values of ≥ 90 ml/min/1.73m², 60 – 89 ml/min/1.73m², 30–59 ml/min/1.73m², 15-29 ml/min/1.73m² and <15ml/min/1.73m² was interpreted as normal, mild, moderate, severe and kidney

failure respectively. Renal function impairment in this study was defined as eGFR <60 ml/min/1.73m² (21).

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20 software program. Descriptive statistics, Chi-square test and Pearson's product were used to test the significance of the results at 95% confidence interval.

Results:

Of the 200 participants recruited in the study, 100 were HIV-infected patients on HAART (case) while 100 were HIV negative (control) subjects. There were 18 males and 82 females in the case group (M:F ratio of 1:4.5) while there were 46 males and 54 females in the control group (M: F ratio of 1:1.2).

Majority of the case subjects were in the age group 36-50 years (55%) while for the control, majority (58%) were in the 20-35 years age group. Most of the HIV-infected patients (55%) were on the TDF+3TC+EFV HAART regimen and 36% had been on therapy for duration of 5-10 years (Table 1).

Table 1: Demographic and clinical characteristics of the study population

Characteristics		HAART treated group (%) (n=100)	HIV negative control (%) (n=100)
Gender	Male	18 (18)	46 (46)
	Female	82 (82)	54 (54)
Age group (years)	20 – 35	14 (14)	58 (58)
	36 – 50	55 (55)	22 (22)
	51 – 65	31 (31)	20 (20)
1 st line ARV therapy	TDF+3TC+EFV	55 (55)	NA
	AZT/3TC+EFV	3 (3)	NA
	AZT/3TC/NVP	11 (11)	NA
2nd line ARV therapy	AZT+3TC+ATZ/r	6 (6)	NA
	TDF/3TC+ATV/r	11 (11)	NA
Duration of therapy	≤ 4 years	29 (29)	NA
	5 – 10 years	36 (36)	NA
	> 10 years	35 (35)	NA

TDF+3TC+EFV = Tenofovir+ Lamivudine+ Efavirenz; AZT/3TC+EFV = Zidovudine+ Lamivudine+ Efavirenz; AZT/3TC/NVP= Zidovudine+ Lamivudine+ Nevirapine; AZT+3TC+ATZ/r = atazanavir/ritonavir; TDF/3TC+ATV/r = Tenofovir+ Lamivudine+ atazanavir/ritonavir; NA= Not Applicable

Table 2a: Elevated transaminase activity in HIV-infected HAART treated and HIV-negative subjects

Patient status	Number (%) with elevated AST activity (>40 U/L)	X ²	p value	Number (%) with elevated ALT activity (>40U/L)	X ²	p value
HAART treated (n=100)	26 (26)	3.068	0.0798 ^{ns}	20 (20)	1.823	0.1170 ^{ns}
HIV negative (n=100)	15 (15)			12 (12)		
Total (n=200)	41 (20.5)			32 (16)		

AST = Aspartate Amino Transferase; ALT = Alanine Amino Transferase; X²= Chi square: ns=not significant

Table 2b: Elevated transaminase activity in HIV-infected HAART treated and HIV-negative participants relative to gender

Category/Parameter	Elevated AST activity		OR	p value	Category/Parameter	Elevated ALT activity		OR	p value
	Male (%)	Female (%)				Male (%)	Female (%)		
HAART treated (n=26)	5 (18.2)	21 (81.8)	0.2083	0.0376*	HAART-treated (n=17)	8 (47.1)	9 (52.9)	0.2222	0.0759 ^{ns}
HIV negative (n=15)	8 (53.3)	7 (46.7)			HIV-negative (n=15)	12 (80)	3 (20)		
Total (n=41)	13 (31.7)	28 (68.3)			Total (n=32)	20 (62.5)	12 (37.5)		

AST=Aspartate Amino Transferase; ALT=Alanine Amino Transferase; HAART=Highly Active Anti-retroviral Therapy; HIV=Human Immunodeficiency Virus; *=significant difference; ns=no significant difference; OR=Odds Ratio

Table 2c: Elevated transaminase activity in HIV-infected HAART treated and HIV-negative participants relative to age group

Category/parameter/age group (years)	*Elevated AST activity			X ²	p value	**Elevated ALT activity			X ²	p value
	20-35 (%)	36-50 (%)	51-65 (%)			20-35 (%)	36-50 (%)	51-65 (%)		
HAART treated	6 (23.1)	11 (42.3)	9 (34.6)	1.040	0.5944 ^{ns}	9 (45)	7 (35)	4 (20)	7.003	0.0301*
HIV negative	4 (26.7)	4 (26.7)	7 (46.7)			2 (16.7)	2 (16.7)	8 (66.6)		
Total	10 (24.4)	15 (36.6)	16 (39.0)			11 (34.4)	9 (28.1)	12 (37.5)		

AST = Aspartate Amino Transferase; ALT = Alanine Amino Transferase; HAART = Highly Active Anti-retroviral Therapy; HIV = Human Immunodeficiency Virus; + = no of patients with elevated AST activity is 41; ++ = no of patients with elevated ALT activity is 32; X² = Chi square; *=significant difference; ns = no significant difference;

As shown in Table 2a, 26% of the 100 HAART-treated HIV-infected patients had elevated serum AST activity (>40 U/L) which was not significantly different from 15% of 100 HIV-negative control ($p=0.0798$). Similarly, 20% of the 100 HAART-treated HIV-infected patients had elevated serum ALT activity that was also not significantly different from 12% of 100 HIV-negative control ($p=0.1770$). The elevated AST activity with respect to gender in the HAART treated patients was significantly different from the HIV-negative control, with more females (81.8%) than males (18.2%) having elevated values in the HAART-treated group ($p=0.0376$) as distinct from 46.7% for females and 53.3% for males in the HIV-

negative control (Table 2b). However, for elevated ALT activity, there was no significant difference with respect to gender between HAART-treated and HIV-negative patients ($p=0.0759$).

With respect to age group as shown in Table 2c, there was statistically significant difference ($p=0.0301$) in the elevated ALT activity between HAART-treated and HIV-negative patients, with those in age group 20-35 years more affected (45%) in the HAART-treated group and those in age group 51-65 years more affected in HIV-negative group (66.6%). However, there was no significant difference in the elevated AST activity with respect to age group in both category of patients ($p=0.5944$).

Table 3a: Elevated serum creatinine, proteinuria and abnormal eGFR in HAART treated and HIV negative subjects

Patient status	Number (%) with elevated creatinine	X ²	p value	Number (%) with proteinuria	X ²	p value	Number (%) with eGFR<60ml/min/1.73m ²	X ²	p value
HAART treated (n=100)	25 (25)	10.751	0.001*	5 (5)	0.7659	0.0886 ^{ns}	24 (24)	2.632	0.1048 ^{ns}
HIV negative (n=100)	7 (7)			7 (7)			14 (14)		
Total (n=200)	32 (16)			12 (6)			38 (19)		

AST = Aspartate Amino Transferase; ALT = Alanine Amino Transferase; HAART = Highly Active Anti-retroviral Therapy; HIV = Human Immunodeficiency Virus; *statistically significant; ns = not significant

Table 3b: Classification of renal status based on eGFR among the HAART -treated patients by gender and age group

Renal status	Gender			p value*	Age group (years)			Total	p value*
	Male (%)	Female (%)	Total		20-35 (%)	36-50 (%)	51-65 (%)		
Normal	7 (38.9)	13 (15.9)	20	0.5502 ^{ns}	6 (42.9)	10 (18.2)	4 (12.9)	20	0.1783 ^{ns}
Mild impairment	8 (44.4)	48 (58.5)	56		6 (42.9)	34 (61.8)	16 (51.6)	56	
Moderate impairment	3 (16.7)	21 (25.6)	24		2 (14.2)	11 (20)	11 (35.5)	24	
Severe impairment	0	0	0		0	0	0	0	
Kidney failure	0	0	0		0	0	0	0	
Total	18	82	100		14	55	31	100	

*p value was calculated by comparing subjects with eGFR >60ml/min/1.73m² with those with eGFR <60ml/min/1.73m²; ns=not significant

Table 3c: Classification of renal impairment based on eGFR among the HIV-negative patients by gender and age group

Renal status	Gender			p value*	Age group (years)			Total	p value*
	Male (%)	Female (%)	Total		20-35 (%)	36-50 (%)	51-65 (%)		
Normal	18 (39.1)	18 (33.3)	36	0.1591 ^{ns}	25 (43.1)	6 (27.3)	5 (25)	36	0.0103 ^s
Mild impairment	19 (41.3)	31 (57.4)	50		28 (48.3)	14 (63.6)	8 (40)	50	
Moderate impairment	7 (15.2)	5 (9.3)	12		5 (8.6)	2 (9.1)	5 (25)	12	
Severe impairment	1 (2.2)	0	1		0	0	1 (5)	1	
Kidney failure	1 (2.2)	0	1		0	0	1 (5)	1	
Total	46	54	100		58	22	20	100	

*p value was calculated by comparing subjects with eGFR >60ml/min/1.73m² with those with eGFR <60ml/min/1.73m²; s=significant; ns=not significant

Table 3a shows the number of subjects (case and control) with elevated serum creatinine level, proteinuria and abnormal eGFR (<60ml/min/1.73m²). Serum creatinine was elevated in 25% of patients in the HAART-treated group, which was significantly higher than 7% in the HIV negative group ($p=0.0010$). The eGFR was abnormal (<60ml/min/1.73m²) in 24% of patients in the HAART-treated group, but this was not significantly higher than 14% in the HIV-negative group ($p=0.1048$). Similarly, the prevalence of 5% for proteinuria in HAART-treated group was not significantly different from 7% in the HIV-negative control group ($p=0.08865$).

Tables 3b and 3c show the classification of renal impairment in relation to gender and age group in both HAART-treated and HIV-negative patients. In the HAART-treated group, renal impairment (eGFR <60ml/min/1.73m²) was higher in females (25.6%), but this was not significantly different ($p=0.5502$) from males (16.7%), and also there was no significant difference with respect to age group (Table 3b). In the HIV-negative group, there was no significant difference ($p=0.1591$) in the occurrence of renal impairment (eGFR <60ml/min/1.73m²) between males (19.6%) and females (9.3%) but the occurrence was significantly higher ($p=0.0103$) in age group 51-65 years (Table 3c).

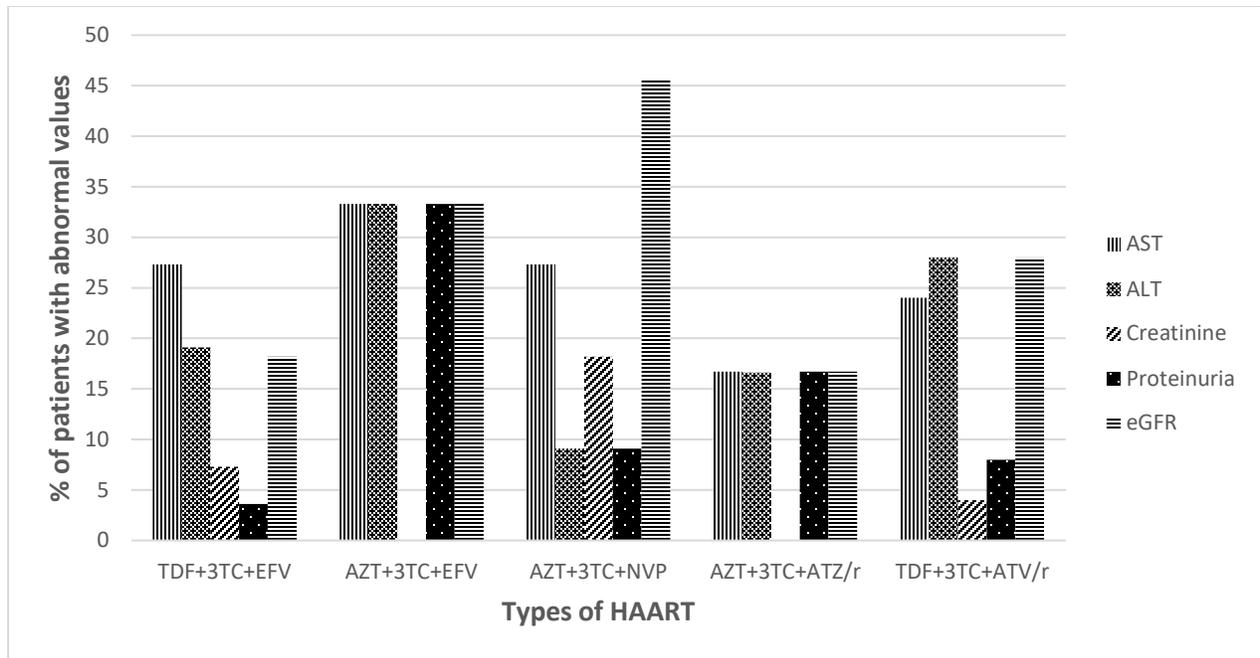


Fig 1: Effect of different types of HAART treatment on markers of liver and kidney function

AST = Aspartate Transaminase; ALT = Alanine Transaminase; TDF+3TC+EFV = Tenofovir+ Lamivudine+ Efavirenz; AZT/3TC+EFV = Zidovudine+ Lamivudine+ Efavirenz; AZT/3TC/NVP = Zidovudine+ Lamivudine+ Nevirapine; AZT+3TC+ATZ/r = atazanavir/ritonavir; TDF/3TC+ATV/r = Tenofovir+ Lamivudine+ atazanavir/ritonavir; eGFR = estimated Glomerular Filtration Rate;

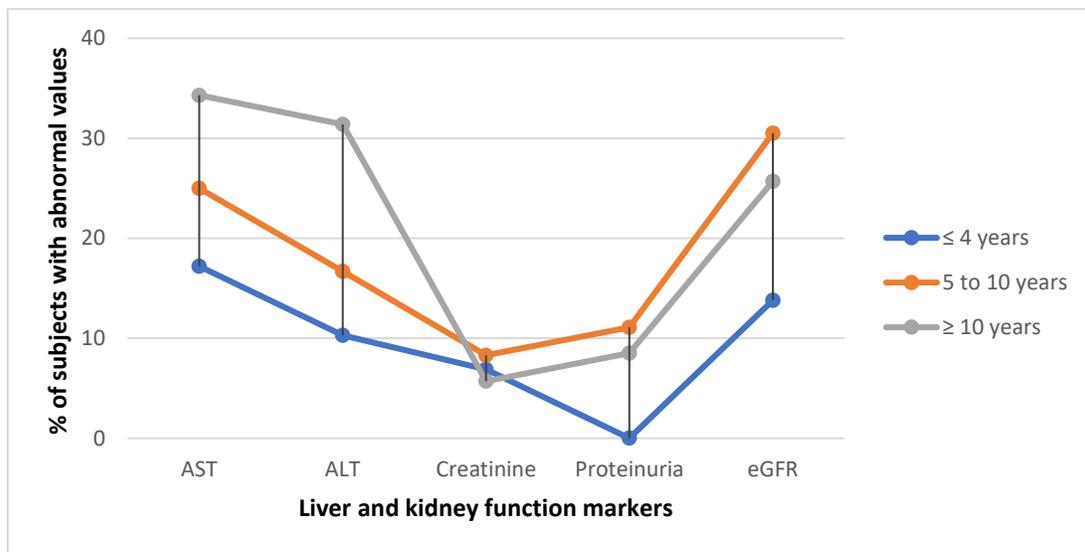


Fig 2: Levels of markers of liver and kidney function and duration of treatment

AST = Aspartate Transaminase; ALT = Alanine Transaminase; eGFR = estimated Glomerular Filtration Rate

Elevated AST and ALT activity occurred more frequently in HAART-treated patients on AZT+3TC+EFV while abnormal eGFR occurred more frequently in those on AZT+3TC+NVP (Fig 1). The prevalence of hepatotoxicity was higher in patients who have been on HAART for over 10

years (34.3% had elevated AST and 31.4% had elevated ALT) while renal impairment (elevated creatinine and abnormal eGFR) was higher in those who have been on HAART for 5 to 10 years (Fig 2).

Discussion:

The overall prevalence of transaminitis among HIV-infected patients on HAART in our study was 26% for AST and 20% for ALT. Although this prevalence was not significantly higher than 15% and 12% respectively for HIV-negative control subjects, our finding is similar to that of a study conducted in northwest Ethiopia by Melashu et al., (16) who reported a prevalence of 20.1% for liver enzyme abnormality in HAART-treated individuals. Megan et al., (17) reported that many studies have shown that HIV infects a wide range of non-hematopoietic cells, including cells of the liver. Destruction of the hepatocytes will be reflected in an upsurge in serum liver enzyme activities. This could possibly explain the elevated liver enzymes observed in the HIV-infected HAART treated subjects in this study.

Our study showed that females in the HAART-treated group had significantly elevated serum AST activity (81.8%) compared to HIV negative group (46.7%) which is consistent with a study conducted in Ghana (18), although that of ALT activity was not significantly related to gender. But serum ALT activity was significantly elevated in age group 20-35 years (45%) in the HAART-treated group, which contradicts the report of a study conducted in Yaoundé, Cameroon, where transaminase levels were highest in HAART experienced patients above the age of 50 years (19). Meanwhile among the HIV-negative participants, ALT was significantly elevated in the age group 51-65 years, which may be attributed to the process of aging with gradual alteration of hepatic structure and function as well as various changes in liver cells (20).

In this study, the prevalence of elevated serum creatinine among patients in the HAART-treated group was 25% while the prevalence in HIV-negative control was 7% ($p=0.0010$). The prevalence of renal impairment in the HAART-treated group, defined as eGFR $<60\text{ml/min}/1.73\text{m}^2$ using the Cockcroft-Gault formula was 24%, while the prevalence was 14% in HIV-negative group ($p=0.1048$). This rate in HAART-treated patients in our study is higher than the rate of 18.2% reported in southeast Ethiopia (21), 21% in northwest Ethiopia (22), 5.5% in Lesotho (23), 6% in Uganda (24) and 1.1% in Tanzania (25). This is in spite of the fact that only subjects with eGFR $<60\text{ml/min}/1.73\text{m}^2$ were considered to have renal impairment in our study, similar to the study by Yewulsew et al, (21). The variation in the rate compared to other studies may be related to differences in population studied, study design, sample size,

and formula/definition used to classify renal impairment (21).

Notably, the prevalence rate of renal impairment among HIV-negative subjects was also high at 14% though not significantly different from the HAART-treated group ($p=0.1048$). The statistically similar prevalence rate of renal impairment in HAART-treated and HIV-negative subjects in our study indicate that HAART therapy is effective in preventing HIV-related renal disease, as this rate was not different from the normal population. The capacity of HAART to reduce viral load, thereby reducing opportunistic infections and improving immunologic response of HIV-infected patients, may account for the non-significant difference in the prevalence of renal impairment in these patients from normal population. In Cameroon, all newly diagnosed HIV patients are immediately placed on HAART as recommended by the World Health Organization, action that we believe has had a great impact in reducing HIV related renal pathologies.

The prevalence of abnormal eGFR ($<60\text{ml/min}/1.73\text{m}^2$) was higher (though not statistically significant, $p=0.5502$) in females (25.6%) than males (16.7%) in the HAART-treated group, which contradicts the report of Yewulsew et al., (21). This may suggest that the female gender is more predisposed to HAART-induced renal toxicity. However, the number of females recruited into the HAART-treated arm of our study was higher, with a male to female ratio of 1:4.5. Molu et al., (26) reported twice females than males in a study that recruited 712 HIV-infected patients in Cameroon. The disparity in the male to female ratio may be attributed to the increased feminization of the HIV epidemic, with large number of females living with AIDS. Again, most women are screened for HIV during pregnancy as part of the pre-natal examination. Aside this, there is also the possibility of increased compliance to treatment by the female gender.

Characteristically, the prevalence of renal impairment was higher in patients aged 51-65 years in both HAART-treated groups (35.5%) and HIV-negative subjects (35%). The two patients with severe renal impairment ($n=1$) and kidney failure ($n=1$) in the study, who were actually among the HIV-negative subjects, were older than 50 years of age thus supporting the phenomenon that renal function decreases with age, with the older age being a risk factor in the general population (27).

Our results showed that most of the patients with elevated transaminase levels were on first-line HAART, which is mostly tenofovir-

based. This was also observed in patients who had renal impairment according to eGFR less than 60ml/min/1.73m² (24). This situation may be due to the direct effect of the tenofovir-based HAART on the liver and kidneys (20), and is contrary to reports of some studies that have documented higher incidence of elevated transaminase activity when protease inhibitors were used (27,28). However, the finding of increase serum ALT and AST activities in HAART treated patients is in agreement with previous studies which reported a characteristic increase in liver transaminases as a result of administration of ART on HIV-infected patients (29).

Patients who have been on treatment duration for longer periods of time had higher prevalence of renal impairment and liver abnormalities in our study. These results are in line with those obtained in a retrospective review of more than 10,000 adults living with AIDS in Boston by Spengler et al., (30), who confirmed that long time antiretroviral therapy is associated with high rate of severe hepatotoxicity regardless of drug class or combination.

One limitation in our study is the unequal ratio of gender and age groups of the HAART-treated group and the HIV-negative control. This was influenced by the study population which was largely made of females and who equally consented to participate in the study. However, to minimize this selection bias, we calculated percentages based on number of participants of either the same gender or the same age group between the cases and controls.

Conclusion:

In conclusion, we report statistically significant elevation in serum activity of transaminases and serum level of creatinine in HIV-infected patients treated with HAART but no significant difference in the prevalence of renal impairment in these patients compared to HIV-negative population. We recommend that after initiation of HAART, liver enzymes and markers of kidney functions should be used in monitoring patients for signs of hepatic or renal toxicity so that the treatment can be adjusted accordingly.

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Original Article

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***In vitro* assessment of the potency of some Newcastle disease vaccine brands in Ibadan, Nigeria**

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Abstract:

Background: Newcastle disease (ND) is a very common and economically important disease of poultry. There is no drug for treatment of the disease during an outbreak in poultry flocks, and prevention by vaccination is one of the recommended control measures. However, post vaccination outbreaks have been observed on many occasions in chicken flocks and one of the causes has been attributed to possible failure of vaccine to confer immunity. This study was designed to evaluate the potency of ND vaccines available in Ibadan, Nigeria.

Methodology: Haemagglutination (HA) technique and elution phenomenon were employed to evaluate the potency of ND vaccines randomly selected in Ibadan. A total of 45 vaccines comprising 9 brands and 5 different strains were selected for potency test. The vaccine brands included 'Vireo 116' (n=10), 'ABIC' (n=5), 'Biovac' (n=9), 'Nobilis' (n=3), 'NVRI' (n=12), 'R2B' (n=2), 'BAL-ND' (n=2), 'Forte dodge' (n=1) and 'Jovac' (n=1), while the vaccine strains in the brands included Lasota, B1, Clone, Komarov, Hitcher, and an unknown strain.

Results: Thirty-five of the 45 (77.8%) ND vaccines tested had more than 4 HA titer (>64) and were therefore regarded as potent. All the 15 (100%) ND Lasota vaccine strain, 7 out of 10 (70%) ND Komarov strain, 4 out of 5 (80%) ND clone and 5 out of 8 (62.5%) ND B1 strains were potent. None of the ND brand 'R2B' vaccine as well as Hitcher strain from 'Nobilis' brand was potent, but all 5, 2, 1 and 1 vaccines tested from brands 'ABIC', 'BAL-ND', 'Fort dodge' and 'Jovac' respectively were potent. Similarly, 9 of 10, 6 of 9, 2 of 3 and 9 of 12 vaccine strains tested from brands 'Vireo 116', 'Biovac', 'Nobilis' and 'NVRI' were respectively potent.

Conclusion: The occurrence of ND vaccines that are not potent in this study may be contributing to post vaccination failure. It is advisable to subject vaccines to potency test before use.

Key words: *in vitro*, assessment, potency, Newcastle disease, vaccine brands, vaccine strains

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Évaluation *in vitro* de la puissance de certaines marques de vaccins contre la maladie de Newcastle à Ibadan, Nigéria

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Abstrait:

Contexte: La maladie de Newcastle (ND) est une maladie très courante et économiquement importante des volailles. Il n'existe aucun médicament pour le traitement de la maladie lors d'une épidémie dans des troupeaux de volailles, et la prévention par vaccination est l'une des mesures de contrôle recommandées. Cependant, des flambées post-vaccination ont été observées à de nombreuses reprises dans des troupeaux de poulets et l'une des causes a été attribuée à un éventuel échec du vaccin à conférer l'immunité. Cette étude a été conçue pour évaluer la puissance des vaccins contre la MN disponibles à Ibadan, au Nigéria.

Méthodologie: La technique d'hémagglutination (HA) et le phénomène d'élution ont été utilisés pour évaluer la puissance des vaccins contre la MN sélectionnés au hasard à Ibadan. Un total de 45 vaccins comprenant 9 marques et 5 souches différentes ont été sélectionnés pour le test d'activité. Les marques de vaccins comprenaient 'Vireo 116' (n=10), 'ABIC' (n=5), 'Biovac' (n=9), 'Nobilis' (n=3), 'NVRI' (n=12), 'R2B' (n=2), 'BAL-ND' (n=2), 'Forte dodge' (n=1) et 'Jovac' (n=1), tandis que les souches vaccinales des marques comprenaient Lasota, B1, Clone, Komarov, Hitcher et une souche inconnue.

Résultats: Trente-cinq des 45 vaccins contre la MN testés (77,8%) avaient plus de 4 titres en HA (>64) et étaient donc considérés comme puissants. Toutes les 15 (100%) souches de vaccin ND Lasota, 7 souches sur 10 (70%) ND Komarov, 4 sur 5 (80%) clones ND et 5 sur 8 (62,5%) souches ND B1 étaient puissantes. Aucun des vaccins 'R2B' de marque ND ni la souche Hitchner de la marque 'Nobilis' n'étaient puissants, mais tous les vaccins 5, 2, 1 et 1 testés des marques 'ABIC', 'BAL-ND', 'Fort dodge' et 'Jovac' respectivement était puissant. De même, 9 des 10, 6 des 9, 2 des 3 et 9 des 12 souches vaccinales testées des marques 'Vireo 116', 'Biovac', 'Nobilis' et 'NVRI' étaient respectivement puissantes.

Conclusion: La présence de vaccins contre la MN qui ne sont pas puissants dans cette étude peut contribuer à l'échec post-vaccinal. Il est conseillé de soumettre les vaccins à un test de puissance avant utilisation.

Mots-clés: *in vitro*, évaluation, puissance, maladie de Newcastle, marques de vaccin, souches vaccinales

Introduction:

Newcastle disease (ND) is a highly contagious viral disease of domestic and wild birds. The disease is caused by the Newcastle disease virus (NDV) of the avian paramyxovirus 1 (APMV-1), a single-stranded, negative-sense ribonucleic acid (-ssRNA) virus belonging to the family Paramyxoviridae, sub-family Paramyxovirinae and genus Rubulavirus (1,2,3). The virus exhibits hemagglutinin/neuraminidase (HN) and fusion (F) glycoprotein spikes at its surface (2,4). These proteins are important in determining the virulence and infectivity of the virus (2). Also, the hemagglutination property is an important aid in the laboratory for virus detection by hemagglutination test (5).

Newcastle disease was first recognized in Indonesia and England in 1926 (6), though earlier existence of the disease dating back to the mid 19th century had been postulated (7). Since then it has become worldwide in distribution (2). It was first reported in Nigeria in 1951 with laboratory confirmation in 1953 (8). Since its recognition, the disease has been regarded as endemic in Nigeria. The disease have been reported as the most important viral disease of poultry in the world (9), and a

major constraint to the poultry industry in Nigeria and Africa in general (9,10), in which it can cause devastating losses in both commercial and village chickens (11).

The disease is characterized by digestive, respiratory and/or nervous signs. The disease has a number of strains that differ in the severity of their clinical signs, ranging from in apparent infection to a rapidly fatal condition (2,6,12). There are many ND vaccines suitable for use in commercial poultry, many of which are available in Nigeria. However, these vaccines can be broadly grouped into two; the first are live/attenuated ND vaccines which are vaccines made with strains of virus of low to moderate virulence that are able to infect cell, but have been substantially modified to lose pathogenicity while maintaining immunogenicity. They are given by intraocular, intranasal, oral and intramuscular routes, and they mirror natural infection and induce cell mediated, humoral and local immunity. The second are killed/inactivated ND vaccines, which are inactivated organisms usually combined with an oil emulsion or aluminium hydroxide adjuvant. They are usually administered to individual birds by intramuscular or subcutaneous injection, and mainly elicit hum-

oural immunity (5,13).

It has been reported that procedure for the production of ND vaccine may differ from one producer to the other in terms of cultures and degree of passage. While some use cell culture for propagation, others may use chicken embryo. Some producers may have short passage while others may perform long passage for vaccine seed attenuation, and this may have effect on potency of the resulting vaccine. From literature on ND vaccines, it has been observed that titres of infective dose vary from one brand to the other, which again could have effect on the potency of the vaccine. Fluctuations in storage temperature of vaccines may also have deleterious effect on vaccine potency. In view of unstable electricity power supply in Nigeria, it is most appropriate to investigate how potent are vaccines sold at various outlets in Ibadan. ND outbreaks in vaccinated flocks are common occurrence in poultry flocks and the causes of such vaccine failure are many, one of which is lack or loss of potency of vaccine. This study was designed to determine the potency of selected ND vaccines available to poultry farmers at the point of purchase in Ibadan.

Materials and method:

Study setting

The study was carried out at the Biotechnology Laboratory of the Department of Veterinary Medicine, University of Ibadan.

Sources of vaccines and materials for testing

Newcastle disease vaccines (a total of 45 vaccines with 9 brands and 5 known strains) were randomly obtained from National Veterinary Research Institute branch office at Mokola, Ibadan, Nigeria, and from private veterinary shops and clinics where poultry vaccines are sold. The materials used included samples of nine brands of ND vaccines, phosphate buffered solution (PBS) tablets, Chicken red blood cells (Crbc), 50 μ l single and 8-channel pipette, distilled water, and U-bottom microtitre plates.

Determination of haemagglutination (HA) titre

First, 0.5% of washed Chicken RBC (Crbc) was prepared in PBS. Then, each vaccine was diluted with PBS to give 250 doses

per ml of PBS. The wells (1-12) in rows of the microtitre plates were filled with 50 μ l of PBS and 50 μ l of the diluted vaccine was added to the PBS in the first well (well 1) in the row and diluted serially up to well 11. Well 12 served as negative control. 50 μ l of 0.5% Crbc was then added to each well 1 to 12. The reaction was allowed to continue until Crbc in the control well 12 completely settles (buttoning). Haemagglutination titre was taken as the reciprocal of the dilution number of last well to show 100% haemagglutination (complete carpeting of well by red blood cells). Each vaccine was tested in triplicate.

Haemagglutination inhibition test

Vaccines that showed haemagglutination were reacted with standard serum containing ND antibodies obtained from chicken challenged with ND vaccine. Inhibition of the haemagglutination was shown by settling of Crbc

Interpretation of results

Observation of complete haemagglutination indicated presence of potent haemagglutinating substance in the vaccine, and the haemagglutination titer indicated potency of the vaccine. Inhibition of the haemagglutination by standard ND serum confirms ND vaccine virus.

Elution test:

The last well to show haemagglutination for each vaccine brand during the haemagglutination test was observed for the period of time it takes for the Crbc to settle. Any vaccine in which haemagglutination disappears, resulting in Crbc settling, has elution. Any vaccine without elution after 30 minutes was regarded as potent and the shorter the complete elution time, the less potent the vaccine

Results:

A total of 45 ND vaccines were tested from the 9 vaccine brands (Nos 1-9), 35 of which belong to 5 different vaccine strains (Lasota, B1, Clone, Komarov and Hitcher) while 6 were unknown vaccine strain (Table 1). Thirty-five of the 45 (77.8%) ND vaccines tested had more than 4 HA titer (>64) and were therefore regarded as potent. All 15 ND

Table: Haemagglutination titre of Newcastle disease vaccines tested for potency

Brand designation	Vaccine brand	Strains	Haemagglutination titre	Score (%)	Elution before 30mins	Verdict
1	Vireo 116: (n=10)	Lasota	512, 128 and 1025	100	None Yes (1/2)	Very potent
		B ₁	64, 512	50		Fairly potent
		Lasota	512, 256, 512, 256 and 512	100		Very potent
2	ABIC: (n=5)	B ₁ Lasota	2048, 128 and 128 128 and 128	100 100	Yes (1/3)	Potent Potent
3	Biovac: (n=9)	B ₁ Clone Lasota	0, 2 and 256 512, 256, 2048 and 64 128 and 128	33.3 75 100		Barely potent Potent Potent
4	Nobilis (n=3)	Hitchner	2	0		Not potent
		Lasota	256	100		Potent
		Clone	256	100		Potent
5	N.V.R.I (Vom): (n=12)	Komarov	256, 256, 640, 128, 256, 16, 64, 16, 512, and 512	70		Fairly potent
		Lasota	256 and 1024 0 and 8	100 0	Yes	Potent Not potent
6	R ₂ B: (n=2)	Not known	128 and 512	100		Potent
7	BAL-ND: (n=2)	Not known	512	100		Potent
8	Fort dodge: (n=1)	Not known	1024	100		Potent
9	Jovac: (n=1)	Not known				

n = number of vaccines tested

Lasota (100%), 7 out of 10 ND Komarov (70%), 4 out of 5 ND clone (80%) and 5 out of 8 ND B₁ strains (62.5%) were potent. None of the ND brand 'R₂B' vaccine as well as Hitchner from 'Nobilis' brand was potent, but all 5, 2, 1 and 1 vaccines tested from brands 'ABIC', 'BAL-ND', 'Fort dodge' and 'Jovac' respectively were potent. Similarly, 9 of 10, 6 of 9, 2 of 3 and 9 of 12 vaccines tested from brands 'Vireo 116', 'Biovac', 'Nobilis' and 'NVRI' were respectively potent (Table).

Discussion:

In Nigeria and elsewhere, live and inactivated vaccines are routinely used in the prevention of Newcastle disease (14). Newcastle disease vaccines are produced by different manufacturers which are sold to veterinarians under different brand names. The number of vaccines tested in this study is a reflection of the availability of each brand of vaccine and may also be a reflection of their popularity, the locally produced vaccine 'NVRI' (26.7%) being the most readily available, followed by 'Vireo 116' (22.2%) and 'Biovac' (20%) brands. The use of HA technique for evaluation of ND vaccine potency is well established (15,16). Ten of the 45 vaccines

(22.2%) tested were not potent, having 4 HA titer or less. The use of such vaccine flocks will result in vaccine failure with consequent risk of ND outbreak.

All ND vaccine Lasota and clone strains tested (100%), 8 of 10 (80%) ND vaccine Komarov strain and 5 of 8 (62.5%) ND vaccine B₁ strains were potent but none of two of the 'R₂B' brand of Komarov strain equivalent was potent. Therefore, in terms of potency reliability, ND vaccine Lasota and clone strains are most reliable. In view of the fact that some of the other strains are not potent, it will be advisable to subject these to potency test before use.

With regard to performance of brands, all strains of ND vaccines of brands 'Vireo 116' and 'ABIC' were potent and therefore are the best brands of choice followed by brand of 'NVRI', which is a local vaccine. The occurrence of ND vaccines that are not potent in this study is worrisome as this may be contributing to post vaccination failure as reported by earlier workers (6,14,17). Ramakrishnan et al., (1) and Okwor et al., (18) reported a gradual but sharp decline in the potency of ND Lasota vaccine when stored under conditions of irregular power supply. The vaccines that were not potent in this study

could have been partly caused by storage under irregular power supply.

Haemagglutination is a measure of the ability of a virus to attach to host cells. This is used to evaluate potency. Elution is an additional observatory phenomenon used to measure the weakness of the virus. Newcastle disease virus that elute earlier than 30mins is usually regarded as weak and therefore not capable of initiating immunological reaction. In this study, three of the vaccines tested were observed to elute before thirty minutes of haemagglutination.

Conclusion:

It is concluded from this study that some commercially available ND vaccines in Ibadan may not be potent as a result of very low HA titer and elution of the vaccine virus. It is therefore recommended that potency test should be carried out on representative ND vaccines before use to prevent vaccination failure.

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**Original Article****Open Access*****In vivo* anti-malarial activity of propranolol against experimental *Plasmodium berghei* ANKA infection in mice***¹Adeyemi, O. I., ¹Ige, O. O., ¹Akanmu, M. A., and ²Ukponmwan, O. E.¹Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria²Department of Medical Pharmacology and Therapeutics, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria*Correspondence to: wadeyemi01@yahoo.com & isaacon@oauife.edu.ng**Abstract:****Background:** Malaria is a mosquito-borne infectious disease caused by *Plasmodium* spp, which is widespread in tropical and subtropical regions of the world. The objective of this study is to evaluate *in vivo* antimalarial activity of propranolol against experimental *Plasmodium berghei* ANKA (PbA) infection in a mouse model.**Methods:** A total of 36 mice weighing between 15 to 18g were randomly divided into six groups of six mice each. Mice in the first group (SAL) were non-infected with *P. berghei* but received normal saline (control), second group (PbA) were mice infected without treatment (control), third group (PRL) were non-infected mice treated with propranolol at the dose of 7.5 mg/kg/bid, fourth group (PbA+PRL) were mice infected and treated with same dose of propranolol, fifth group (QUN) were non-infected mice treated with quinine at a dose of 20 mg/kg stat, then 10 mg/kg bid, and sixth group (PbA+QUN) were infected mice treated with quinine. Parasitaemia, physiological conditions (cognitive function, temperature) and lethality of infected mice were monitored over 7-day period to assess the antimalarial activity of propranolol and quinine. The Y-maze paradigm was used to assess cognitive impairment induced by PbA infection. The effects of propranolol on malaria indices and cognitive impairment were compared with that of quinine and the control using T-test statistical method.**Results:** Mortality of mice at day 7 in the infected group without treatment (PbA) was 100% (6/6) while mortality was 50% (3/6) in infected group treated with propranolol (PbA+PRL) and 33.3% (2/6) in infected group treated with quinine (PbA+QUN) (OR=2.000, $p=1.000$). No mortality was recorded in any of the three groups of uninfected mice. Propranolol reduced parasitaemia to a trough level of 1.40 ± 0.07 three days after treatment, comparable to trough level of 1.39 ± 0.0633 by quinine but did not reverse PbA-induced hypothermia, which quinine did.**Conclusion:** Propranolol demonstrated *in vivo* antimalarial activity against experimental PbA infection in mice comparable to that of quinine.**Keywords:** malaria, propranolol, quinine, *Plasmodium*, cerebral malaria

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Activité antipaludique *in vivo* du propranolol contre l'infection expérimentale par *Plasmodium berghei* ANKA chez la souris*¹Adeyemi, O. I., ¹Ige, O. O., ¹Akanmu, M. A., et ²Ukponmwan, O. E.¹Département de pharmacologie, Faculté de pharmacie, Université Obafemi Awolowo, Ile-Ife, Nigéria²Département de pharmacologie médicale et thérapeutique, Collège des sciences de la santé, Université Obafemi Awolowo, Ile-Ife, Nigéria*Correspondance à: wadeyemi01@yahoo.com & isaacon@oauife.edu.ng**Abstrait:****Contexte:** Le paludisme est une maladie infectieuse transmise par les moustiques causée par *Plasmodium* spp, qui est répandue dans les régions tropicales et subtropicales du monde. L'objectif de cette étude est d'évaluer l'activité

antipaludique *in vivo* du propranolol contre une infection expérimentale à *Plasmodium berghei* ANKA (PbA) dans un modèle murin.

Méthodes: Un total de 36 souris pesant entre 15 et 18 g ont été réparties au hasard en six groupes de six souris chacun. Les souris du premier groupe (SAL) n'étaient pas infectées par *P. berghei* mais ont reçu une solution saline normale (contrôle), le deuxième groupe (PbA) était des souris infectées sans traitement (contrôle), le troisième groupe (PRL) était des souris non infectées traitées par propranolol à la dose de 7,5mg/kg/bid, le quatrième groupe (PbA+PRL) étaient des souris infectées et traitées avec la même dose de propranolol, le cinquième groupe (QUN) étaient des souris non infectées traitées avec de la quinine à une dose de 20mg/kg stat, puis 10mg/kg bid et le sixième groupe (PbA+QUN) étaient des souris infectées traitées avec de la quinine. La parasitémie, les conditions physiologiques (fonction cognitive, température) et la létalité des souris infectées ont été surveillées sur une période de 7 jours pour évaluer l'activité antipaludique du propranolol et de la quinine. Le paradigme du labyrinthe en Y a été utilisé pour évaluer les troubles cognitifs induits par l'infection au PbA. Les effets du propranolol sur les indices du paludisme et les troubles cognitifs ont été comparés à ceux de la quinine et du témoin à l'aide de la méthode statistique du test T.

Résultats: La mortalité des souris au jour 7 dans le groupe infecté sans traitement (PbA) était de 100% (6/6) tandis que la mortalité était de 50% (3/6) dans le groupe infecté traité avec du propranolol (PbA+PRL) et 33,3% (2/6) dans le groupe infecté traité par la quinine (PbA+QUN) (OR=2.000, $p=1.000$). Aucune mortalité n'a été enregistrée dans aucun des trois groupes de souris non infectées. Le propranolol a réduit la parasitémie à un niveau minimum de $1,40 \pm 0,07$ trois jours après le traitement, comparable au niveau minimum de $1,39 \pm 0,0633$ de la quinine, mais n'a pas inversé l'hypothermie induite par le PbA, ce que la quinine a fait.

Conclusion: le propranolol a démontré une activité antipaludique *in vivo* contre l'infection expérimentale au PbA chez la souris comparable à celle de la quinine.

Mots-clés: paludisme, propranolol, quinine, *Plasmodium*, paludisme cérébral

Introduction:

Malaria is a parasitic disease caused by protozoan parasites of the genus *Plasmodium*, which is transmitted by female anopheles' mosquitoes. Only five plasmodia species develop in humans, these are *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium knowlesi* and *Plasmodium malariae*. Of these, only *Plasmodium vivax* and *Plasmodium ovale* have persistent liver forms that may lead to relapses after the initial blood infection. However, *P. falciparum* malaria is the main species of public health importance as a result of its lethality and virulence (1).

Cerebral malaria is the most severe neurologic complication of infection caused by *P. falciparum* (2) and collectively involves the clinical manifestations of malaria that induce changes in mental status and coma. It presents as acute widespread infection of the brain accompanied by fever (3). Even though this type of malaria is most common in children living in sub-Saharan Africa, it should be considered in any patient with impaired consciousness that has recently travelled to a malaria endemic area. Cerebral malaria is a major cause of acute non-traumatic encephalopathy in tropical countries with high mortality, and over the past two decades, the extent of persistent neuro-cognitive deficits after recovery has become very apparent (4).

Cerebral malaria is fatal within days if left untreated, immediate treatment is therefore crucial (5). Because natural immunity to malaria is not fully understood and thus cannot yet be artificially imitated by drugs, control and

prevention strategies are significant, two of which include anti-malarial chemotherapy and adjunctive measures. Chemotherapy for cerebral malaria now primarily involves the use of artesunate (6), and if not available, artemether or quinine can be given as alternatives (7). However, in most health facilities, quinine still finds great use especially because it is still safe in the first trimester of pregnancy. Adjunctive measures for cerebral malaria include the use of anti-pyretics such as paracetamol and anti-convulsants such as the benzodiazepines.

The mature human erythrocyte is a terminally differentiated cell that lacks sub-cellular organelles such as nucleus or secretory structures, de-novo protein or lipid biosynthesis, and does not endocytose its plasma membrane (8). Parasite entry into erythrocytes is a complex, dynamic process, but it has been shown that Gs peptide signaling is involved and β -adrenergic blockers inhibit the signaling process *in vitro* (9). It has been documented that propranolol, an antagonist of G protein-coupled β -adrenergic receptors, dampens Gs activity in erythrocytes (10). This prevents the invasion and growth of the parasite in the erythrocyte and hence easy clearance by the immune system.

Meanwhile, cerebral malaria has been attributed to be a major cause of cognitive impairment in sub-Saharan Africa (11). This impairment has been postulated to be due to engorgement of brain microvasculature as a result of parasites movement into the brain (12). *Plasmodium berghei* ANKA (PbA) infection mimics human cerebral malaria and this strain of plasmodium is widely used in the study of

diffuse encephalopathy arising from cerebral malaria (13,14).

The objectives of this study are to assess *in vivo* anti-malarial activity of propranolol by monitoring end stage symptoms (hypothermia, high parasitaemia and lethality) and to evaluate the effects of propranolol on cognitive impairment, in a mouse model experimental PbA infection.

Materials and method:

Experimental animal study

Locally bred apparently healthy albino mice of both sexes, 6 to 8 weeks old, with weights ranging between 15 g to 18 g were used. The animals were obtained from and initially housed in a room in the animal house of the Department of Pharmacology, Faculty of Pharmacy of the Obafemi Awolowo University, Ile-Ife. The animals were housed in plastic and metal cages that were regularly cleaned and beddings changed. The animals were maintained on regular commercial animal feed diet and drinking water provided *ad libitum*.

Experimental drugs

The experimental drugs used were Quinine hydrochloride (Laborate Pharmaceuticals, India) and Propranolol (BDH chemicals), which were administered intraperitoneally.

Preparation of PbA parasite inoculum

The rodent malaria parasite used was a quinine sensitive strain, *Plasmodium berghei* ANKA (PbA). The parasite was obtained from the Institute of Medical Research and Training, University College Hospital (UCH), Ibadan, and is used as a model to mimic *Plasmodium falciparum* that causes cerebral malaria in human (13,14). A standard inoculum of 10^6 parasitized erythrocytes was prepared by dilution of blood harvested from a donor mouse with normal saline by serial persaging.

Experimental study protocol

The animals (total of 36 mice) were allowed to acclimatize to their new environment before randomly dividing them into six groups of six mice each. In each group, the mice were weighed and marked for identification. The first group (SAL) comprised non-infected (control) mice which received normal saline, the second group (PbA) comprised infected (control) mice without treatment, the third group (PRL) comprised non-infected mice treated with propranolol at the dose of 7.5 mg/kg/bid (10), the fourth group (PbA+PRL) comprised infected mice treated with propranolol, the fifth group (QUN) comprised non-infected mice treated

with quinine at a dose of 20 mg/kg stat, then 10 mg/kg bid (15), and the sixth group (PbA+QUN) comprised infected mice treated with the same dose of quinine.

The experimental study was conducted over a period of 7 days while cognitive functions of the mice were assessed on day 8. Mice were infected on day 0 of the study by intraperitoneal administration of the standard inoculum of the parasitized erythrocytes. Propranolol and quinine were administered starting from day 5 to day 7, as cerebral malaria is known to develop on day 5 of PbA infection in mice (14,16). The non-infected mice were also treated with propranolol and quinine to assess their effects on cognitive functions of the mice in the absence of parasitaemia.

Assessment of PbA induced lethality in mice and effects of propranolol and quinine

The lethality of PbA infection was determined by the number of infected mice that died in the untreated (PbA) and those treated with propranolol (PbA+PRL) and quinine (PbA+QUN).

Assessment of PbA induced hypothermia and effects of propranolol and quinine

Hypothermia, which is infection induced observation in mice seen as an end stage symptom (16), was determined by measuring rectal temperature of mice with an electronic thermometer daily for each group during the period of the study

Assessment of PbA induced cognitive impairment and effects of propranolol & quinine

The Y-maze, a measure of short-term memory (17), was used to assess cognitive function in the mice. Each animal was dropped in one of the arms of the maze. The movement of the mouse into other arms was then observed and recorded. The assessment was done for six (6) minutes each. The Y-maze experiment was done for each group before infection and after experimental treatment.

Assessment of PbA parasitaemia and effects of propranolol and quinine

Parasitaemia was obtained by preparing slides from thin blood smears made from the tail of the mice, fixing with methanol and staining with Giemsa. Slides for the parasites were prepared for the three groups of mice infected with PbA. The stained slide of the blood smear was mounted on a binocular microscope and a drop of immersion oil applied to the slide (15). The 100x objective lens (with total magnification of 1000 x) of the micro-

scope was used to examine a slide field for parasitized and non-parasitized red blood cells. In each field, the number of parasitized red blood cells was counted, and the total number of red blood cells was determined. The slides were prepared daily for each group during the period of the study.

Statistical analysis

The percentage parasitaemia in each field was calculated as; % Parasitaemia = number of parasitized cells/total number of cells (x100). For each slide, 7 fields were counted and the average of the 7 fields was calculated as the average % Parasitaemia (total % parasitaemia/7). The T-test was used to compare percentage parasitaemia between the infected groups while Fisher Exact was used to compare differences in mortality rate

between groups of treated mice. A *p* value less than 0.05 was considered significant.

Results:

Effects of propranolol and quinine on lethality of infected mice

Table 1 shows the lethality of PbA infection on the mice in treated and untreated groups. Mortality of mice at day 7 in the infected group without treatment (PbA) was 100% (6/6) while mortality was 50% (3/6) in the infected group treated with propranolol (PbA+PRL) and 33.3% (2/6) in the infected group treated with quinine (PbA+QUN). There was no mortality of mice in uninfected group that received saline (SAL), propranolol (PRL) and quinine (QUN) throughout study period.

Table 1: Number of surviving animals in the uninfected and infected (with and without treatment) groups of mice

Day	No of surviving mice in groups of mice					
	PbA	SAL	PbA+PRL	PRL	PbA+QUN	QUN
Day 3	6	6	6	6	6	6
Day 4	6	6	6	6	6	6
Day 5	3	6	5	6	5	6
Day 6	1	6	4	6	4	6
Day 7	0	6	3	6	4	6

PbA = *Plasmodium berghei* ANKA; SAL = Normal Saline; PRL = Propranolol; QUN = Quinine; PBA+PRL = *Plasmodium berghei* ANKA and Propranolol; PBA+QUN = *Plasmodium berghei* ANKA and Quinine.

Table 2: The average percentage parasitaemia in the three experimentally infected groups of mice with and without treatment

DAY	% Parasitaemia PbA	% Parasitaemia PbA+PRL	% Parasitaemia PbA+QUN
Day 3	6.53±0.3971	6.67±0.1512	6.80±0.1219
Day 4	7.91±0.0734	8.23±0.0618	7.90±0.1247
Day 5	9.37±0.1770	4.53±0.2072**	4.12±0.0872**
Day 6	11.78±0.0000	2.78±0.2148**	3.06±0.1777**
Day 7	+0	1.40±0.0651**	1.39±0.0633**

*Significantly different from control at *p* < 0.05; **Significantly different from control at *p* < 0.01
 PBA - *Plasmodium berghei* ANKA, PBA+PRL - *Plasmodium berghei* ANKA + Propranolol, PBA+QUN - *Plasmodium berghei* ANKA + Quinine
 The average parasitaemia was calculated for the surviving mice on day 5 upward, the parasitaemia for mouse in PbA group on day 6 was the parasite count of the only one surviving mouse in the group (Table 1). + = no surviving mouse on day 7

Parasitaemia in infected mice

Table 2 shows the average percentage parasitaemia in the three experimentally infected groups of mice with and without treatment. Infected mice without treatment (PbA) showed increasing parasitaemia, with mortality (50%) occurring on day 5 (average parasitaemia of 9.37 ± 0.1770) and 100% mortality on day 7. Infected mice treated with propranolol (PbA+PRL) on day 5 showed a statistically significant decrease in average parasitaemia of mice on day 5 (4.53 ± 0.2072) from that of day 4 (8.23 ± 0.0618) ($p < 0.05$), with mortality of 16.7% (1/6) and the lowest parasitaemia was recorded on day 7 (1.40 ± 0.0651), with mortality of 50% (3/6).

Infected mice treated with quinine (PbA+QUN) also showed statistically significant decrease in average parasitaemia on day 5 (4.12 ± 0.0872) from average parasitaemia on day 4 (7.90 ± 0.1247), with mortality of 16.7% (1/6), and the lowest parasitaemia was recorded on day 7 (1.39 ± 0.0633), with mortality of

33.3% (2/6).

Rectal temperature in infected and non-infected mice

Mice infected with PbA showed reduction in rectal temperature as the infection progressed compared to the uninfected groups (SAL, PRL and QUN) in which the temperature did not show any significant variation throughout the study period ($p > 0.05$) (Table 3). Infected mice treated with propranolol showed an increase in rectal temperature from the day of treatment as the reduction in rectal temperature caused by PbA infection from day 3 was reversed by day 5 propranolol treatment on days 6 and 7 of the study.

Similarly, infected mice treated with quinine showed an increase in rectal temperature from the day of treatment as the reduction in rectal temperature caused by PbA infection from day 3 was reversed by quinine treatment on days 5, 6 and 7 of the study.

Table 3: Mean of rectal temperature of uninfected and infected (with and without treatment) groups of mice

Day	Mean temperature in groups of mice					
	SAL (°C)	PBA (°C)	PRL (°C)	PBA+PRL (°C)	QUN (°C)	PBA+QUN (°C)
Day 0	36.51±0.1428	36.73±0.0799	36.80±0.2039	37.17±0.2348	36.73±0.0732	36.50±0.3678
Day 1	36.69±0.1378	36.75±0.1675	36.99±0.2651	36.79±0.4344	36.90±0.1188	36.29±0.3475
Day 2	36.90±0.1152	36.94±0.0221	36.55±0.1886	35.26±0.2263**	36.58±0.1755	35.42±0.0641**
Day 3	36.70±0.2563	35.23±0.1030**	36.98±0.1575	35.05±0.1258**	36.59±0.1868	34.93±0.0771**
Day 4	36.73±0.1145	34.43±0.0844**	36.56±0.1121	33.95±0.1367**	36.59±0.1670	33.40±0.0949**
Day 5	36.54±0.1485	32.40±0.2000**	36.72±0.0760	34.48±0.1190**	36.40±0.0949	34.49±0.0886
Day 6	36.70±0.1057	32.00±0.0000**	36.57±0.1301	35.12±0.2252	36.69±0.9610	35.32±0.2278
Day 7	36.57±0.1605	+0	36.62±1.0955	36.00±0.1528	36.69±0.1177	36.08±0.1652

**Significantly different from control at $p < 0.01$; *Significantly different from control at $p < 0.05$; (n=6, mean \pm SEM); SAL – Normal Saline, PBA – *Plasmodium berghei* ANKA, PRL – Propranolol, PBA+PRL – *Plasmodium berghei* ANKA and Propranolol, QUN – Quinine, PBA+QUN – *Plasmodium berghei* ANKA and Quinine. Average rectal temperature was calculated for the surviving mice on day 5 upward, the rectal temperature for mouse in PbA group on day 6 was the rectal temperature of the only one surviving mouse in the group (Table 1). + = no surviving mouse on day 7

Table 4: Cognitive functions of uninfected and infected (treated and non-treated) groups of mice

Day	SAL	PbA	PRL	PbA+ PRL	QUN	PbA +QUN
Day 0	61.67±8.6923	74.39±4.6644	56.38±4.4795	73.68±8.3500	64.43±1.8524	74.39±5.8723
Day 8	74.90±5.242	+0	82.97±7.5013	47.62±9.9143*	67.03±5.2421	57.43±9.7463

*Significantly different from control at $p < 0.05$; (n = 6, mean \pm SEM); SAL – Normal Saline, PBA – *Plasmodium berghei* ANKA; PRL – Propranolol, PBA+PRL – *Plasmodium berghei* ANKA and Propranolol, QUN – Quinine, PBA+QUN – *Plasmodium berghei* ANKA and Quinine. + = no surviving mouse on day 7

Effects of propranolol and quinine on cognitive functions of infected and uninfected mice

The cognitive functions of PbA infected untreated mice could not be assessed as 100% mortality had occurred on day 7 (Table 4). Expectedly, there was no significant change in the cognitive functions of the un-infected mice that received normal saline. Propranolol (PRL) and quinine (QUN) had no effect on the cognitive function of un-infected mice as there was no significant difference between the cognitive functions before and after treatment.

However, there was significant decrease ($p < 0.05$) in the cognitive functions in infected mice treated with propranolol (PbA+PRL), indicating that propranolol did not reverse the impaired cognitive function caused by PbA infection. In contrast, there was no significant difference ($p > 0.05$) in the cognitive function of infected mice treated with quinine, indicating that quinine protected the mice from impaired cognitive function caused by PbA infection.

Discussion:

In this study, the lethality of *P. berghei* ANKA (PbA) infections in infected untreated mice from 50% on day 5, 83.3% on day 6 and 100% on day 7 confirms this parasite to be virulent. This parasite is a laboratory model that is often used to mimic *P. falciparum* infection which causes cerebral malaria in human (13,14). Lethality can be used as a measure of anti-malarial activity of a drug. In this study, propranolol reduced mortality in infected mice by 50% on day 7 implying that although propranolol is effective, its use for treatment of cerebral malaria should be initiated as soon as possible (5). In comparison, quinine reduced mortality of infected mice by 66.7% on day 7 which confirms the established antimalarial property of quinine, especially against cerebral malaria (7,15). Propranolol reduced the increasing parasitaemia in infected mice when

treated was initiated on day 5 of the study, which further confirms its anti-malarial property, and this was comparable to the antimalarial property of quinine (15).

The use of rectal temperature as a parameter is borne out of the fact that one of the end stage manifestations of cerebral malaria is hypothermia (18). In the group infected with the parasite, there was a marked significant decrease in the rectal temperature which confirms the establishment of PbA infection and induced hypothermia from cerebral affectation. Our study demonstrated that propranolol reversed the hypothermia induced by PbA infection with recovery of some of the mice, and this action was comparable to that of quinine on the infected mice as previously established (15).

The Y-maze is generally acceptable as a model to assess cognitive function (17). Although cognitive function of the mice in the PbA infected group could not be assessed because there was 100% mortality of the mice on day 7, impaired cognitive function, which we believed was caused by the PbA infection in the mice as previously reported (11,12), was not reversed by propranolol treatment. This was demonstrated by significant reduction in the cognitive function of the infected mice in spite of the propranolol treatment. Propranolol and quinine also did not show any effect on cognitive functions of uninfected animals which may imply their safety. However, quinine appears to protect the infected mice from impaired cognitive function as the cognitive function of the PbA infected mice was not significantly reduced upon treatment with quinine. This protective quinine phenomena has similarly been described for *P. falciparum* cerebral malaria in humans (11).

Conclusion:

This study demonstrated anti-malarial property of propranolol in an *in vivo* mice

experiment with comparable antimalarial property to that of quinine, a widely used anti-malarial drug.

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**Original Article****Open Access****Prevalence and factors associated with dengue fever among febrile patients attending secondary health facilities in Kano metropolis, Nigeria**

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Abstract:

Background: The Nigeria Centre for Disease Control (NCDC) has categorized dengue fever as a priority epidemic-prone disease. Kano metropolis is a hub for international trade and has seen rapid population growth with unplanned urbanisation. This provides the right environment for dengue virus transmission and spread. Most fevers in Nigeria and Kano in particular are treated as malaria or typhoid. This study aimed to determine the prevalence and factors associated with dengue fever and dengue-malaria co-infection among febrile patients in Kano metropolis.

Methodology: We conducted a hospital-based cross-sectional study among febrile patients attending secondary health facilities in Kano metropolis. We used multistage sampling technique to recruit 440 participants into the study. An interviewer administered structured questionnaire was used to collect participants' information, while blood was screened for malaria using rapid diagnostic test (RDT) kit and tested for dengue fever using an ELISA kit for dengue IgM. Data was cleaned and analysed using Microsoft Excel 2016 and Epi Info version 7.2 to calculate frequencies, proportions and odds ratios.

Results: The median age of participants was 24 years (IQR= 13-36 years) while the age-group most represented was the 10-19 years' age group with 97 (22.9%) participants. Males constituted 257 (60.6%) while most were single 238 (56.1%). Dengue IgM was positive for 332 (78.3%), while malaria RDT was positive for 81 (19.1%) and 67 (15.8%) were positive for both. Those aged above 25 years were less likely to present with dengue fever (OR=0.6; 95% CI=0.33-0.97). Dengue fever contributes a high percentage to febrile illnesses seen in Kano metropolis. Significant proportions of febrile patients have both dengue and malaria co-infection. None of the participants had dengue as a presumptive diagnosis.

Conclusion: These findings imply dengue fever should feature as a prominent differential for febrile illnesses and dengue screening tests should be made available for all cases seen in Kano.

Keywords: seroprevalence; dengue fever; RDT; secondary health facilities; Nigeria

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Prévalence et facteurs associés à la dengue chez les patients fébriles fréquentant les établissements de santé secondaires de la métropole de Kano, au Nigéria

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Abstrait:

Contexte: Le Nigeria Center for Disease Control (NCDC) a classé la dengue parmi les maladies à tendance épidémique prioritaires. La métropole de Kano est une plaque tournante du commerce international et a connu une croissance démographique rapide avec une urbanisation imprévue. Cela fournit le bon environnement pour la transmission et la propagation du virus de la dengue. La plupart des fièvres au Nigeria et à Kano en particulier sont traitées comme le paludisme ou la typhoïde. Cette étude visait à déterminer la prévalence et les facteurs associés à la dengue et à la co-infection dengue-paludisme chez les patients fébriles de la métropole de Kano.

Méthodologie: Nous avons mené une étude transversale en milieu hospitalier auprès de patients fébriles fréquentant des établissements de santé secondaires de la métropole de Kano. Nous avons utilisé une technique d'échantillonnage en plusieurs étapes pour recruter 440 participants dans l'étude. Un questionnaire structuré administré par un intervieweur a été utilisé pour collecter les informations des participants, tandis que le sang a été testé pour le paludisme à l'aide d'un kit de test de diagnostic rapide (TDR) et testé pour la dengue à l'aide d'un kit ELISA pour les IgM de la dengue. Les données ont été nettoyées et analysées à l'aide de Microsoft Excel 2016 et d'Epi Info version 7.2 pour calculer les fréquences, les proportions et les rapports de cotes.

Résultats: L'âge médian des participants était de 24 ans (IQR=13-36 ans) tandis que le groupe d'âge le plus représenté était le groupe d'âge 10-19 ans avec 97 (22,9%) participants. Les hommes en constituaient 257 (60,6%) tandis que la plupart étaient célibataires 238 (56,1%). Les IgM de la dengue étaient positives pour 332 (78,3%), tandis que les TDR du paludisme étaient positifs pour 81 (19,1%) et 67 (15,8%) étaient positifs pour les deux. Les personnes âgées de plus de 25 ans étaient moins susceptibles de présenter une fièvre dengue (OR=0,6; IC à 95%=0,33-0,97). La dengue contribue à un pourcentage élevé des maladies fébriles observées dans la métropole de Kano. Des proportions significatives de patients fébriles présentent une co-infection à la fois par la dengue et le paludisme. Aucun des participants n'avait de dengue comme diagnostic présumé.

Conclusion: Ces résultats impliquent que la dengue devrait apparaître comme un différentiel important pour les maladies fébriles et des tests de dépistage de la dengue devraient être disponibles pour tous les cas observés à Kano.

Mots-clés: séroprévalence; la fièvre de la dengue; RDT; établissements de santé secondaires; Nigeria

Introduction:

Dengue fever (DF), one of the Neglected Tropical Diseases (NTDs), accounts for about 96 million (95% CI 67-136 million) clinically apparent infections annually (1). It affects large populations especially in the tropics and subtropics (2). The World Health Organization (WHO) designated it "a disease that may constitute public health emergency of international concern with implication for health security" (3). Unfortunately, it is under-recognized and often misdiagnosed because of similarities in the presenting features with malaria and typhoid fever which are more easily diagnosed (4). Infected humans and monkeys are the reservoirs of the virus to uninfected *Aedes* mosquitoes. Treatment is by early supportive management since no antiviral agent is available. The first dengue vaccine, Dengvaxia, was registered in Mexico in December, 2015 (5).

The *Aedes* vector has wide distribution in Africa enabling extensive transmission of the virus (4), but records of dengue occurrence in Africa is poor (1). However, dengue-like illnesses do occur but unfortunately are misdiagnosed (2). Documentation of dengue

has come mostly from individual sero-surveys or from diagnosis in travellers returning from Africa and from outbreak investigations. Available data shows that dengue is endemic in 34 countries from all regions of Africa (4), and in the 50 years from 1960 to 2010, twenty laboratory-confirmed dengue outbreaks were reported in 15 countries, mostly from East Africa (4). Bhatt et al., in 2013 (1), using a formal modelling framework to map the global distribution of dengue risk, estimated there to be about 64.1 million dengue infections in Africa annually, of which 15.7 million manifests clinically.

Due to poor surveillance and lack of reporting in Nigeria (6), limited local prevalence studies have been the source of information on dengue magnitude (7-10). The Nigeria Centre for Disease Control (NCDC) has categorized dengue fever as a priority epidemic-prone disease (11). Anecdotal data show increasing number of patients being managed in Kano for malaria with standard treatments ending with poor outcomes like death and loss of consciousness in adults. These cases could be DF or its severe form. Kano metropolis is a hub for international trade and has seen rapid population growth with unplanned urban-

zation. It has a lot of slums with open drainages, sewages, open empty containers (12), poor waste management (13) and plenty of ponds providing the right environment for *Aedes*. With no policy for routine detection, it is important to know whether some of the febrile cases with severe presentation are DF but missed because they are not looked for. We aimed to determine the proportion of patients presenting with fever who have DF and dengue-malaria co-infection at secondary health facilities in Kano metropolis and the proportion of missed diagnosis among febrile patients.

Materials and method:

Study setting, design and population

We conducted a hospital-based cross-sectional study in November 2017 among febrile patients attending General Outpatient Departments (GOPD) of secondary health facilities in Kano metropolis. Kano metropolis is made of eight local government areas (LGA) with 12 secondary health facilities.

Sample size and sampling technique

We used a dengue-malaria co-infection prevalence of 43% (14) and assumed a non-response rate of 15% (considering the fact that blood samples will be taken and the possibility of decline or of lysed samples) and obtained a minimum sample size of 440. We used multistage sampling technique to recruit patients into the study. In the first stage, two LGAs were selected out of the eight that make up Kano metropolis by balloting. In the second stage, we selected one hospital by balloting from the list of secondary health facilities located in each of the LGAs selected above; Waziri Shehu Gidado General Hospital (WSGH) in Ungogo (with monthly average number of febrile patients of 1980) and Murtala Muhammad Specialist Hospital (MMSH) in Kano municipal (this hospital serves like a referral centre for the less privileged because a lot of services are free and has a particularly high monthly average turnover of febrile patients of 4200).

The sample size was allocated to the hospitals proportionate to the average monthly turnover of febrile patients, thus WSGH had 141 participants while MMSH had 299. In the third stage, we used a sampling interval of 14 to systematically recruit patients into the study in each of the participating hospitals until the proportion of the sample size allocated to the hospital was achieved. A total of 440 patients were recruited for the study.

Inclusion and exclusion criteria

We included patients with fever $\geq 38^{\circ}\text{C}$

in the last two weeks prior enrolment, who were being tested for malaria or typhoid fever. Patients with jaundice, children under 6 months of age or patients with fever from other known causes such as upper or lower respiratory tract infections, urinary tract infections and bacterial sepsis were excluded.

Data collection

An interviewer administered, pre-tested, structured questionnaire was used to collect information on sociodemographic characteristics, signs and symptoms of the current illness, household and environmental factors.

Sample collection

Whole blood was collected from each subject, a portion of which was used to screen for malaria using rapid diagnostic test (RDT) kit (SD Bioline), and the other portion was then separated and serum stored at -20°C until tested for DF IgM using an Enzyme Linked Immunosorbent Assay (ELISA) test kit (DeMeditec). This test is a qualitative immunoenzymatic determination of specific antibodies based on the ELISA technique. After the preparatory step involving dilution of the serum samples, the diluted samples were added to microplates which have been coated with specific antigens to bind corresponding antibodies in the sample.

After washing the wells to remove all unbound sample materials, a horseradish peroxidase (HRP) labelled conjugate was added, which binds to the captured antibodies. Following a second washing step, the unbound conjugate was removed. The immune complex formed by the bound conjugate was subsequently visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product was proportional to the amounts of specific antibodies in the sample. The reaction was stopped by adding sulphuric acid producing a yellow colour at endpoint. Absorbance was read at 450 nm using an ELISA microwell plate reader within 30min of stopping the reaction. All absorbance readings were subjected to the test validation criteria as provided by the kit manufacturer, then converted to international units (U) before interpretation as positive, negative or indeterminate. All indeterminate results were categorized as negative as repeat sample collection was not possible.

Statistical analysis

Data collected were cleaned and analysed using Microsoft Excel 2016 and Epi Info 7.2. Univariate analyses were presented as frequencies and proportions in tables. Relationship between DF and sociodemographic (low educational status included those with no formal education, primary or secondary edu-

cation), household, and environmental factors were examined and presented as odds ratios, with their 95% confidence intervals and *p* value. We used binary logistic regression model to determine independent predictors of DF.

Ethical approval

Ethical approval was obtained from the operational research committee of the Kano State Ministry of Health (KSMOH) with reference number MOH/Off/797/T.1/499. Written informed consent was sought from the participants, and where participants were less than 10 years old, consent was sought from their caregivers. For participants 10-14 years of age, parental consent and individual assent were obtained. Failure to give consent or opting out of the study did not negatively affect client's management. Participants were notified of their malaria status to take back to their physicians for further management, while results of dengue status which became available much later were shared with the hospitals in which the study was conducted, and KSMOH to guide change in policy.

Results:

Of the 440 febrile patients recruited,

424 participated fully in all components of the study. Two hundred and fifty-seven (60.6%) were male. The median age was 24 years (IQR =13-36) while the age-group most represented was the 10-19 years with 97 (22.9%) subjects. More than half were single (55.4%). Majority of the patients had attained secondary education 215 (50.7%), while most were either unemployed 223 (52.6%) or students 85 (20.0%) (Table 1).

Dengue IgM was positive in 332 (78.3%), malaria RDT was positive in 81 (19.1%) while both were positive in 67 (15.8%). Almost half of the cases, 197 (46.5%), had malaria as the health providers' preliminary diagnosis (Table 2). The age-group 20-29 years had the highest age-specific dengue IgM prevalence (Table 3). On bivariate analysis, those who were less than 25 years were twice more likely to have dengue infection (OR=2.1; 95%CI=1.29-3.33). Having a house near bush (OR=0.6; 0.36-0.96), and having open water containers around the house (OR=0.5; 0.29-0.74) were also statistically significant but appeared protective (Table 4). Following unconditional logistic regression, none of these factors was an independent predictor of DF (Table 5).

Table 2: Status of dengue IgM among febrile patients and physicians' preliminary diagnosis in Kano metropolis, November, 2017

Variable	Frequency (n)	(%)
Test results (n=424)		
Positive for Dengue IgM only	332	78.3
Positive for Malaria RDT only	81	19.1
Positive for both Dengue and Malaria	67	15.8
Health providers preliminary diagnosis		
Malaria	197	46.5
Malaria and Typhoid	1	0.2
Typhoid	88	20.8
Not provided	138	32.6
Proportion of missed diagnosis		100.0

Table 1: Sociodemographic characteristics of febrile patients in Kano metropolis, November 2017

Characteristics	Frequency (n=424)	%
Age group (years)		
<10	81	19.1
10-19	97	22.9
20-29	83	19.6
30-39	80	18.9
40-49	62	14.6
≥50	21	5.0
Gender		
Female	167	39.4
Male	257	60.6
Marital status		
Married	185	43.6
Single	238	56.1
Widow	1	0.2
Ethnic group		
Fulani	66	15.6
Hausa	355	83.7
Others	3	0.7
Level of education		
No formal education	76	17.9
Primary	115	27.1
Secondary	214	50.5
Tertiary	19	4.5
Occupation		
Student	85	20.0
Civil servant	14	3.3
Business entrepreneur	75	17.7
Retired	8	1.9
Unemployed	223	52.6
Artisans	3	0.7
Others	16	3.8

Table 3: Age-specific prevalence of positive dengue IgM among febrile patients in secondary health facilities in Kano Metropolis in 2017

Age group (years)	Respondents (n=424)	Positive IgM (n=332)	Age specific prevalence IgM (%)
<10	81	67	82.7
10-19	97	80	82.5
20-29	83	69	83.1
30-39	80	59	73.8
40-49	62	43	69.4
50+	21	14	66.7
	424	332	78.3

Table 4: Risk Factors for dengue fever among febrile patients in Kano Metropolis in November 2017

Variables	Dengue Status		Odds Ratio (95% CI)	P-value	
	Positive n (%)	Negative n (%)			
Age group (years)					
	<15	106 (82.2)	23 (17.8)	1	
	15-24	76 (87.4)	11(12.6)	1.5 (0.69-3.26)	0.30
	25+	150 (72.1)	58 (27.9)	0.6 (0.33-0.97)	0.04
Gender					
	Male	202 (78.6)	55 (21.4)	1.1 (0.65-1.67)	0.85
	Female	130 (77.8)	37 (22.2)		
Education					
	Low	146 (76.8)	44 (23.2)	0.9 (0y54-1.37)	0.53
	High	185 (79.4)	48 (20.6)		
Occupational Status					
	Unemployed	260 (77.3)	75 (22.4)	0.8 (0.45-1.47)	0.50
	Employed	72 (80.9)	17 (19.1)		
Travelled out of Kano					
	Yes	77 (77.8)	22 (22.2)	1.0 (0.56-1.80)	0.99
	No	150 (77.3)	43 (22.3)		
House window nets					
	Yes	286 (78.8)	77 (21.2)	1.2 (0.60-2.21)	0.66
	No	45 (76.3)	14 (23.7)		
Sleep under nets					
	Yes	213 (78.3)	59 (21.7)	1.0 (0.61-1.61)	0.96
	No	117 (78.5)	32 (21.5)		
Open gutters/ponds					
	Yes	100 (73.0)	37 (27.0)	0.7 (0.40-1.05)	0.08
	No	225 (80.7)	54 (19.4)		
House near bush					
	Yes	86 (71.7)	34 (28.3)	0.6 (0.36-0.96)	0.03
	No	245 (81.1)	57 (18.9)		
Open water containers					
	Yes	103 (69.6)	45 (30.4)	0.5 (0.29-0.74)	<0.01
	No	228 (83.2)	46 (16.8)		
Spray environment daily					
	Yes	298 (79.5)	77 (20.5)	1.6 (0.79-3.14)	0.20
	No	32 (71.1)	14 (28.9)		
House near a construction site					
	Yes	195 (77.7)	56 (22.3)	0.9 (0.53-1.4)	0.54
	No	130 (80.3)	32 (19.8)		
Waste around the house					
	Yes	135 (75.0)	45 (25.0)	0.7 (0.43-1.12)	0.13
	No	185 (81.1)	43 (18.7)		
Clean Your surroundings					
	Not regularly	1 (33.3)	2 (66.7)	0.1 (0.01-1.44)	0.11
	Regularly	310 (79.5)	80 (20.5)		
Dispose your waste					
	Not regularly	24 (77.4)	7 (22.6)	0.9 (0.39-2.24)	0.87
	Regularly	302 (78.7)	82 (21.4)		
Clean gutters around the house					
	Not regularly	43 (72.9)	16 (27.1)	0.7 (0.37-1.31)	0.26
	Regularly	280 (79.4)	74 (20.6)		

Discussion:

This study found an overall DF IgM positive prevalence of 78.3% among febrile patients. Having IgM in the blood is an indication of recent infection by the dengue virus or less likely cross-reaction from another flavivirus. The prevalence in this study is high. Possible explanations include the fact that this study was done among febrile patients with suggestive symptoms. Hitherto, such patients were treated presumptively as malaria or typhoid, with or without laboratory confirmation. It is possible that dengue has been the cause of fevers that are treated as malaria but go undetected. In this study, the health providers' preliminary diagnosis was malaria

(46.5%), typhoid (20.8%) or not provided at all. None of the participants had dengue as a presumptive diagnosis, which means though DF does not have any specific treatment, patients may not receive any supportive management in line with DF, with increased risk of possible severe disease and complications.

The GOPD of most public secondary health facilities in Kano are patronized predominantly by people in the lower socioeconomic class. Most middle-and-high income earners prefer private and tertiary care health facilities where they can pay to get better services. Zellweger et al., (15) had suggested that dengue is commoner among people of the lower socioeconomic class. This in part could explain the high DF prevalence in this study.

Table 5: Independent risk factors for dengue fever among febrile patients in Kano Metropolis, November, 2017

Risk factors	Adjusted odds ratio	95% Confidence interval	
Age group (years)			
<15	1		
15-24	1.9	0.75	4.94
≥25	1.0	0.50	2.15
House near bush			
Yes	0.6	0.15	2.61
No	1		
Open gutters/ponds			
Yes	1.1	0.34	3.81
No	1		
Open water containers			
Yes	0.6	0.21	1.56
No	1		
Waste around house			
Yes	0.9	0.50	1.75
No	1		
Spray surrounding daily			
Yes	1		
No	2.1	0.86	5.23
Clean surroundings regularly			
Yes	1		
No	0.2	0.02	3.31

Another possible explanation is the fact that this study was carried out in an urban setting. Kano metropolis has seen unplanned urbanization which has been severally identified as one of the risk factors for the increased incidence of DF. When this is associated with low literacy, unemployment and living in densely populated areas where open sewers/gutters, random waste disposal (12,13) are the norm, which are all present in the study areas, it is not surprising that we obtained such high prevalence of DF. The implication of this finding is that DF is highly prevalent in Kano metropolis, and should be considered as a prominent cause of febrile illness.

The prevalence from this study is higher than some other studies done in other parts of Nigeria. Bello et al., (16) working in Kaduna, located in the same region as our study reported an IgM prevalence of 51.9%, although this was lower, one of the three LGAs (Kafanchan) in which the work was carried out had a prevalence of 80.0% (indicating disparity even within the same state), which is similar to the results of the current study. On the other hand, Baba et al., (17) working in the arid north-eastern city of Maiduguri repor-

ted IgM seropositivity of 0.5% which may be due to the extreme hot temperature and low rainfall, denying the mosquitoes the necessary breeding grounds. But another study conducted in the same city among internally displaced people (IDP) camps by Oyinloye et al., (10) reported 74% among antenatal attendees and non-pregnant women and men with febrile complaints. Still in Maiduguri, Idris et al., (18) confirmed that dengue type-3 virus was circulating in the community after obtaining a seropositivity of 10.1%, however this was limited to only one serotype, and the detection method used was culture-based micro-neutralization assay and not IgM ELISA. Adedayo et al., (8) working in Ilorin, north-central Nigeria, reported a prevalence of 30.8%, however, the study was limited to children under five of age.

Other studies from outside northern Nigeria have reported similar seroprevalence rates. In south-western Nigeria, Oyero et al., (14) working in Ibadan reported a prevalence of 35% using ELISA that detects the NS1 antigen. However, this antigen is mainly positive during the first 3-5 days of fever and wanes subsequently, thus this could under-

estimate the prevalence especially where late presentation is the norm. Olufisayo et al., (7) on the other hand, in their study in Ife, reported 25.7% among febrile patients. Still another study in Ogbomosho by Oladipo et al., (19) reported IgM prevalence of 17.2%, but this was conducted among apparently healthy individuals, thus may not be comparable to the current study.

The rates reported in the studies show the disparity that exists between different studies in the prevalence of dengue fever. Some of these could be as a result of differences in detection methods, population sampled, or regional variation. Bhatt et al., (1) in their work surmised that there could be variation as a result of disparity between actual and reported infection numbers because low proportion of patients with apparent infections seek care from formal health facilities. Elsewhere, beyond Nigeria, Mazaba-Liwewe et al., (20) working in Zambia, reported 4.1% IgG seroprevalence rate, while in Sudan, Himatt and colleagues (21) reported a seroprevalence rate of 9.4% but this was also among apparently healthy individuals in a community-based survey.

The prevalence of dengue-malaria co-infection obtained from this study was 15.8%. This finding indicates that co-infection is common, which is not surprising as both infections are endemic in the environment. This study was conducted in the month of November just after the rainfalls season, when there were still pockets of ground water collections, and when temperature is high, giving conducive environment for mosquito breeding. Thus, this allows for the possibility of the two infections coexisting. That this has not been previously reported in Kano may be due to the fact that there may be no desire to look out for the existence of other febrile illnesses, when one disease can be diagnosed in an acute febrile illness. The implication of this is that health workers should no longer be satisfied with having made a diagnosis but should look further in case there are other coexisting conditions, especially where the mode of transmission is the same or similar. It also stresses the need for early diagnosis and management in all cases of acute febrile illnesses.

Oyero et al., (14) reported a dengue-malaria co-infection of 10% in Ibadan, south-western Nigeria, which is not too different from what was obtained from the current study. They also concluded that all malaria patients had positive IgG for dengue, indicating previous infection. Similarly, Olufisayo et al., (7) in Ife reported that out of 25.7% of dengue IgM-positive febrile patients, only 9 (19.6%) had no detectable malaria. Also, Barua et al., (22) in Mumbai, India reported a

coinfection of 10.25% and 6.7% in 2014 and 2015 respectively. However, the co-infection in our study is higher than 3% reported by Rao et al., (23) in Odisha, India.

The only significant factor in this study associated with the risk of developing DF was age group 25 years and above, who were less likely to develop with DF compared to age group less than 14 years. However, this was not an independent predictor of DF. This finding is not too different from that of Toan et al., (24) in a case-control study during an outbreak who reported that morbidity was highest between the ages 15 and 30 years, and drops as age increased. Other factors that appeared significant include having a house near the bush or harbouring open water containers around the house. But these appeared as protective factors rather than risk factors and eventually were not independently related to dengue infection. The absence of any independent risk factor in this study could be due to the fact that most of the participants had similar background of low socioeconomic setting. This is evidenced by majority having low educational status or being unemployed.

The major limitation of this study is the fact that the population is skewed towards the lower socioeconomic class, being the population that mostly patronize the GOPDs of secondary health facilities. Furthermore, the study was conducted in an urban setting.

Conclusion:

We found that high proportion of febrile illnesses seen in Kano metropolis were due to DF. Significant proportion of these co-exist with malaria. None of these cases were suspected by the health providers and were presumed to be malaria, typhoid, or both or simply as fever of unknown cause. These findings have already been shared with the state ministry of health for action. We recommend that dengue RDT Kits should be made available as a screening test for febrile illnesses. There is need to train health providers to suspect and recognize DF in order to institute the right management of febrile illnesses.

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**Short Communication****Open Access****Gastrointestinal parasites among swine bred in Edo State, Nigeria**

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Department of Medical Laboratory Science, School of Basic Medical Sciences,
University of Benin, Benin City, Nigeria*Correspondence to: zainab.omoruyi@uniben.edu**Abstract:**

Background: Swine production in Nigeria is encountering several constraints among which are diseases. This study was designed to determine the prevalence of gastrointestinal parasites of swine with respect to sex, age, location and hygiene practices in the breeding sites in three piggery farms; University of Benin Animal Farm; Osasio Farm Uselu in Egor Local Government Area (LGA), and Ojemai Farm Ekehuanwa Road, Oredo LGA, Benin City, Edo State, Nigeria.

Methods: A total of 150 pigs were sampled from the 3 piggery farms. Faecal sample (10g each) was aseptically collected from the rectum of each selected pig and processed by concentration method to microscopically identify parasites in both saline and iodine preparations. A designed pre-tested structured questionnaire was interviewer-administered to each piggery owner to collect information on husbandry practices, animal health care issues and potential risk factors associated with parasitosis. Data was analysed using SPPSS version 20 software, while odd ratios (OR) with 95% confidence intervals (CI) were calculated on each potential risk factors.

Results: Of the 150 pigs examined, 130 (86.6%) were infected with five gastrointestinal parasitic agents. *Strongyloides ransomi* 81 (54%) was the most frequently identified parasite, followed by *Ascaris suum* 68 (45.3%), *Giardia lamblia* 31 (20.6%), *Entamoeba polecki* 10 (6.6%) and *Trichuris suis* 10 (6.6%). Single and mixed infections were not significantly associated with the observed prevalence ($p>0.05$). Similarly, the sex of pig was not significantly associated with the prevalence of parasites ($p=0.8824$). The prevalence of parasitosis among the grower (87.8%) was not significantly different from the adult swine (87.5%) ($p>0.05$). Osasio had the highest infection rate (90.0%) but this rate was not significantly different from the two other farms ($p>0.05$).

Conclusion: This study confirms high prevalence of gastrointestinal parasitic infections in pigs bred in Edo State, Nigeria. It is therefore recommended that farmers improve on their biosecurity and adhere to routine de-worming regimen of pigs.

Keywords: age, gender, prevalence, gastrointestinal, parasite, swine

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Parasites gastro-intestinaux chez les porcs élevés dans l'État d'Edo, au Nigéria

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Contexte: La production porcine au Nigéria rencontre plusieurs contraintes parmi lesquelles les maladies. Cette étude a été conçue pour déterminer la prévalence des parasites gastro-intestinaux des porcs en fonction du sexe, de l'âge, de l'emplacement et des pratiques d'hygiène dans les sites de reproduction de trois porcheries; Ferme

animale de l'Université du Bénin; Osasio Farm Uselu dans la région du gouvernement local d'Egor (LGA) et Ojemai Farm Ekehuanwa Road, Oredo LGA, Benin City, État d'Edo, Nigéria.

Méthodes: Au total, 150 porcs ont été échantillonnés dans les 3 fermes porcines. Un échantillon fécal (10 g chacun) a été prélevé de manière aseptique dans le rectum de chaque porc sélectionné et traité par une méthode de concentration pour identifier au microscope les parasites dans les préparations salines et d'iode. Un questionnaire structuré pré-testé conçu a été administré par un intervieweur à chaque propriétaire de porcherie pour recueillir des informations sur les pratiques d'élevage, les problèmes de santé animale et les facteurs de risque potentiels associés à la parasitose. Les données ont été analysées à l'aide du logiciel SPSS version 20, tandis que les rapports impairs (OR) avec des intervalles de confiance (IC) à 95% ont été calculés sur chacun des facteurs de risque potentiels.

Résultats: Sur les 150 porcs examinés, 130 (86,6%) ont été infectés par cinq agents parasitaires gastro-intestinaux. *Strongyloides ransomi* 81 (54%) était le parasite le plus fréquemment identifié, suivi par *Ascaris suum* 68 (45,3%), *Giardia lamblia* 31 (20,6%), *Entamoeba polecki* 10 (6,6%) et *Trichuris suis* 10 (6,6%). Les infections simples et mixtes n'étaient pas associées de manière significative à la prévalence observée ($p>0,05$). De même, le sexe du porc n'était pas significativement associé à la prévalence des parasites ($p=0,8824$). La prévalence de la parasitose chez le producteur (87,8%) n'était pas significativement différente de celle des porcs adultes (87,5%) ($p>0,05$). Osasio avait le taux d'infection le plus élevé (90,0%) mais ce taux n'était pas significativement différent des deux autres fermes ($p>0,05$).

Conclusion: Cette étude confirme la forte prévalence des infections parasitaires gastro-intestinales chez les porcs élevés dans l'État d'Edo, au Nigeria. Il est donc recommandé que les éleveurs améliorent leur biosécurité et adhèrent au régime vermifuge de routine des porcs.

Mots-clés: âge, sexe, prévalence, gastro-intestinal, parasite, porcin

Introduction:

Swine production is one of the fastest growing livestock sectors worldwide and forms an integral part of the rural economy in many parts of the world by providing additional animal protein sources for human consumption, generating employment and reducing poverty (1). Swine production has a number of advantages over other livestock sectors, which includes higher and quicker returns on investment, early maturity, short generation interval and smaller space requirements for production (2). Parasitic diseases from infection by protozoans, helminths, and some arthropods have an immense influence on the lives of humans and animals, causing variety of illnesses that could be rapidly fatal in some cases or chronic, incidental or even asymptomatic (3). The major diseases of pigs include Africa swine fever, swine influenza, parasitic and non-infectious diseases. Parasitic diseases are a major obstacle to the growth of the pig industry.

Helminthiasis in pigs is often associated with subclinical infections resulting in poor feed conversion and delayed achievement of market weight (4). The common pig parasites identified in previous studies include *Strongyloides* species, *Ascaris* species, *Trichuris* species, *Entamoeba histolytica* and *Entamoeba coli* (5,6,7). In Nigeria, intestinal parasitism is a debilitating condition in piggery. In spite of this, helminthiasis is often neglected because of its in apparent clinical signs. This branch of livestock industry in developing countries like Nigeria is however, saddled with religious,

cultural, social and environmental challenges as well as infectious diseases (5). Stunted growth and prolonged fertility are often associated with helminthiasis. This is a major setback to efficient, effective and result oriented livestock production (8). The aim of this study is to determine the prevalence of gastrointestinal parasites of swine in selected piggery in Benin City, Edo State, Nigeria.

Materials and method:

Study area

This study was carried out in three different pig farms; University of Benin Animal farm; Osasio farm, Uselu, Egor LGA, and Ojemai farm, Ekehuanwa Road, Oredo LGA, in Benin City, Edo State, Nigeria. Benin City is the capital of Edo State, located in South-south Nigeria. It has an estimated population of 1,147,188 (9). The State relies on trading, private transport system, farming (pig, cattle and goat) from the northern part of Nigeria as one of its sources of protein.

Egor LGA has an area of 93 km² and lies between longitude 5^o 34E and latitude 6^o 23N, with a population of 339,899 (9) and a population density of 70 people/km² with headquarters in Uselu town. It shares borders with Ovia Northeast in Oredo, Ovia Southwest, and Ikpoba-Okha LGAs. Oredo has an area of 249 km² and a population of 295,818 inhabitants by the 2006 census and lies between longitude 5^o 37E and 15^o 26E and latitude 6^o 19N and 58^o 83N.

Study population and ethical approval

A total of 150 pigs were randomly

selected, 50 from each farm. Prior to the conduct of the study, ethical approval was obtained from Ethics and Research Committee, Ministry of Health, Benin City, Edo State, and informed consent was obtained from the swine breeders.

Sample collection and processing

Faecal specimens were aseptically collected in the morning into universal containers from the rectum of piglets, growers and adult swine bred (n=150) in the selected study area. The samples were properly labelled and transported to the laboratory of University of Benin Teaching Hospital, Benin City, Edo State for processing.

The faecal samples were processed using sedimentation technique (10). About 1 g of faecal sample was mixed in 4 ml 10% formal saline, properly mixed and sieved. To the filtrate, 3 ml of diethyl ether was added, mixed and spun at 3000rpm for 3 minutes. The supernatant was discarded and the sediment was resuspended, and examined in both saline and iodine preparations under the light micro-

scope (Nikon, Tokyo, Japan) using 10x and 40x objectives. Eggs were identified on the basis of their morphological features as described by Soulsby (11).

Statistical analysis

The data obtained were analysed using SPSS version 20 software, and odd ratios (OR) with 95% confidence intervals (CI) were calculated for each potential risk factor.

Results:

The overall prevalence rate of gastrointestinal parasites in the study sites was 86.6% (130/150) with prevalence rate of 90% in Osasio, 85% in Ojemai and 84% in University of Benin farm ($p=0.972$) (Table 1). Five gastrointestinal parasites of medical importance were identified; *Strongyloides ransomi* was the predominant intestinal parasite in the study with 54% (81/150) (Table 2).

The overall mixed infection rate was 21.3% with University of Benin farm having the highest mixed infection rate (Table 3). Sow were

Table 1: Frequency distribution of gastrointestinal parasites in relation to location

Location	Number examined	Number infected (%)	<i>p</i> value
Uniben Farm	50	42 (84)	0.972
Ojeimai Farm	50	43 (85)	
Osasio Farm	50	45 (90)	
Total	150	130 (86.6)	

Table 2: Frequency distribution of parasites among swine in Benin City, Edo State, Nigeria

Species	Number positive	% Prevalence
<i>Strongyloides ramsoni</i>	81	54
<i>Ascaris suum</i>	68	45.3
<i>Giardia lamblia</i>	31	20.6
<i>Entamoeba polecki</i>	10	6.6
<i>Trichuris suis</i>	10	6.6

Table 3: Prevalence of single and mixed infections of gastrointestinal parasites in the three different study sites

Site	Number examined	Single infection (%)	Mixed infection (%)	<i>p</i> value
Uniben Farm	50	30 (60)	12 (24)	0.789
Ojeimai Farm	50	32 (64)	11 (22)	
Osasio Farm	50	36 (72)	9 (18)	
Total	150	98 (65.3)	32 (21.3)	

Table 4: Distribution of gastrointestinal parasites in relation to sex of swine

Sex	Number examined	Number infected (%)	Odd ratio (OR)	95% CI	<i>p</i> value
Female (sow)	61	54 (88.5)	0.9646	0.6001 - 1.554	0.8824
Male (boar)	89	76 (85.4)			
Total	150	130 (86.6)			

Table 5: Distribution of gastrointestinal parasites in relation to age of swine

Age (weeks)	Number examined	Number infected (%)	<i>p</i> value
1 - 10 (piglet)	45	38 (84.4)	0.875
11 - 20 (grower)	41	36 (87.8)	
> 20 (adult)	64	56 (87.5)	
Total	150	130 (86.6)	

more infected (88.5%) compared to Boar (85.4%) but the difference was not statistically significant associated (OR=0.9646, 95% CI = 0.6001, 1.554; $p=0.8824$) (Table 4). For age distribution, the growers had a higher prevalence of 87.8% compared to adult 87.5% and piglets 84.4%, though the difference was not statistically significant ($p>0.05$) (Table 5).

Discussion:

This study confirms the reports of previous study on the prevalence of gastrointestinal parasites in swine (12), which indicates that gastrointestinal parasitic infection is one of the major challenges facing breeding pigs in Edo State. The prevalence of 86.6% reported in this study is higher than the rate of 74.8% reported in Makurdi, Benue State (13) and 79.0% in a commercial farm in Abia State, Nigeria (14). This may be attributed to poor sanitary condition, nutritional challenges and poor routine treatment in the

present study. The result of this study is consistent with the reports of Okorafor et al., (12) and Tidi and Ella (5) who also reported high prevalence of similar gastrointestinal parasites in pigs in Nigeria. A higher prevalence (91.0%) than the present findings was reported in Burkina Faso, a West African country like Nigeria (16).

The faecal examination during the study revealed the presence of five common gastrointestinal parasites of swine similar to reports that *Trichuris suis*, *Ascaris suum*, *Strongyloides ransomi* and *Entamoeba polecki* were the most prevalent parasites of swine in a research farm (12). This could be attributed to the extensive system of farm management widely practiced within the study area, lack of veterinary services, irregular deworming observed in the farms, and low income to maintain the farms. Single infection was more prevalent (65.3%) compared to mixed infection (21.3%), though this was not significantly different ($p=0.789$). This is consistent with the study by

Atawalna et al., (15) which reported that single infections were more prevalent in parts of Ghana. The possible explanation to the mixed infection might be the fact that pigs are reared extensively and also their feeding troughs are not cleaned regularly which might aid acquisition of more infective eggs of parasites from contaminated environment.

The results of this study also showed that sex and age of the swine were not significantly associated with infection ($p>0.05$) however sow had a slightly higher prevalence (88.52%) compared to boar (85.4%). This finding agrees with other studies that reported higher prevalence of intestinal parasites in sow than boar in Nigeria (5) and Burkina Faso (16) but disagrees with previous studies that reported higher prevalence in boar than sow (17,18). This could be due to the fact that female pigs are kept much longer for breeding purposes as compared to the male pigs, which are usually fattened and sold off (12). With regards to age, adult pigs recorded the higher prevalence though the prevalence in relation to the infection was not significant. It has been suggested that the confinement of the piglets and growers in the pens tend to reduce their chances of contact with the disease agents.

With respect to location, the prevalence of infection among the pigs in the three farms were high and although the prevalence was slightly higher in Osasio farm, this was not significantly different from the other farms. The high prevalence of infection in all the farms could be the result of common poor management system and types of husbandry practiced in the farms.

In conclusion, this study confirmed the presence of five gastrointestinal parasites that are significant threat to pigs in the study areas. Based on the possibility of transfer of some of these helminths from pigs to man, there is urgent need to develop strategies to control the parasites in pigs.

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**Short Communication****Open Access**

Comparative distribution of bacterial contaminants of packaged and unpackaged polyherbal products sold in Nnewi, Nigeria

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Abstract:

Background: The use of herbal medicine continues to remain popular despite advances in orthodox medicine largely as a result of affordability and availability. However, contaminated and potentially toxic polyherbal preparations remain a public health challenge despite regulations instituted by concerned agencies in Nigeria. The objective of this study was to determine and compare the bacterial contaminants of different polyherbal products sold in Nnewi, Nigeria

Methodology: This study evaluated the bacteriological profile of 22 packaged and 22 unpackaged polyherbal preparations sold in Nnewi, Nigeria. The samples were collected from different herbal medicine shops in Nnewi by simple random sampling and were assayed for comparative bacterial loads with chromogenic media and their total viable counts evaluated following standard method for microbial load analysis.

Results: Bacterial contaminants were isolated from 9 of 22 (40.9%) packaged polyherbal samples while 13 of 22 (59.1%) samples were bacteriologically sterile. For the unpackaged polyherbal, bacterial contaminants were isolated from 18 of 22 (81.8%) samples while 4 of 22 (18.2%) were bacteriologically sterile (OR 0.1538, $p=0.0122$). The most frequently isolated bacterial contaminant in the packaged polyherbal samples was *Enterococcus faecalis* with 33.3% (6/18) while *Salmonella* sp was the least frequently isolated with 5.6% (1/18). For the unpackaged polyherbals, the most frequently isolated bacterial contaminant was *Staphylococcus aureus* with 25% (7/28) while *Salmonella* sp and *E. faecalis* were the least frequently isolated with 10.7% (3/28) each. The median total viable count of the packaged group of the polyherbal products was 1.48×10^6 CFU/ml, while the median total viable count for unpackaged group of polyherbals was 1.95×10^6 CFU/ml.

Conclusion: This study shows that many polyherbal products sold in Nnewi are potentially contaminated with bacterial agents. It is therefore imperative that herbal medicine practitioners be enlightened on hygienic ways of preventing microbial contamination during polyherbal production.

Keywords: Bacterial contaminants, herbal products, Nnewi, Nigeria

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Distribution comparative des contaminants bactériens des produits polyherbal emballés et non emballés vendu à Nnewi, Nigeria

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Abstrait:

Contexte: L'utilisation de la phytothérapie continue de rester populaire malgré les progrès de la médecine orthodoxe en grande partie en raison de l'abordabilité et de la disponibilité. Cependant, les préparations à base de plantes contaminées et potentiellement toxiques restent un problème de santé publique malgré les réglementations mises en place par les agences concernées au Nigeria. L'objectif de cette étude était de déterminer et de comparer les

contaminants bactériens de différents produits polyherbal vendus à Nnewi, Nigeria

Méthodologie: Cette étude a évalué le profil bactériologique de 22 préparations polyherbal emballées et 22 non emballées vendues à Nnewi, Nigeria. Les échantillons ont été collectés dans différents magasins de plantes médicinales de Nnewi par simple échantillonnage aléatoire et ont été analysés pour les charges bactériennes comparatives avec les milieux chromogènes et leurs dénombrements viables totaux évalués selon la méthode standard pour l'analyse de la charge microbienne.

Résultats: Des contaminants bactériens ont été isolés dans 9 des 22 échantillons polyherbal emballés (40,9%) tandis que 13 des 22 échantillons (59,1%) étaient bactériologiquement stériles. Pour le polyherbal non emballé, des contaminants bactériens ont été isolés de 18 des 22 échantillons (81,8%) tandis que 4 des 22 (18,2%) étaient bactériologiquement stériles (OR 0,1538, $p=0,0122$). Le contaminant bactérien le plus fréquemment isolé dans les échantillons polyherbal emballés était *Enterococcus faecalis* avec 33,3% (6/18) tandis que *Salmonella* sp était le moins fréquemment isolé avec 5,6% (1/18). Pour les polyherbals non emballés, le contaminant bactérien le plus fréquemment isolé était *Staphylococcus aureus* avec 25% (7/28) tandis que *Salmonella* sp et *E. faecalis* étaient les moins fréquemment isolés avec 10,7% (3/28) chacun. Le nombre total viable médian du groupe emballé des produits polyherbal était de $1,48 \times 10^6$ UFC/ml, tandis que le nombre total viable médian pour le groupe non emballé de polyherbales était $1,95 \times 10^6$ UFC/ml.

Conclusion: Cette étude montre que de nombreux produits polyherbal vendus à Nnewi sont potentiellement contaminés par des agents bactériens. Il est donc impératif que les praticiens en phytothérapie soient éclairés sur les moyens hygiéniques de prévenir la contamination microbienne pendant la production de polyherbes.

Mots-clés: Contaminants bactériens, produits à base de plantes, Nnewi, Nigéria

Introduction:

Herbal medicine is a medication made from herbs and has long been used as a source of alternative medicines in developed, developing and underdeveloped countries. Throughout the ages, humans have turned to herbal medicine for healing. All societies have folk medicine traditions that include the use of plants and plant products. Many licensed drugs used today in conventional medicine originated from herbal products.

The World Health Organization (WHO) estimates that about 4 billion or at least 80% of the world's population use herbal preparations for some aspects of primary health care (10). In Nigeria, herbal therapy remains a popular alternative in many traditional communities where orthodox medicine is not affordable (2). Herbal medicine practitioners in Nigeria use various herbal preparations to treat various types of ailments including diarrhoea, urinary tract infections, typhoid fever and skin diseases (8). Unfortunately, many of these herbal medicine practitioners do not follow hygienic procedures in preventing microbial contaminants during production of their polyherbal products. The study is designed to comparatively evaluate bacteriological contaminants of packaged and unpackaged polyherbals sold in Nnewi, Nigeria.

Materials and method:

Collection of polyherbal samples

A total of 44 samples of liquid formulations of the polyherbals produced in Nigeria were purchased from 16 different herbal shops and trade-medical hawkers, who were selected by simple random sampling within Nnewi town. The samples of the packaged polyherbal preparations (n=22) were purchased while samples of the unpackaged extemporaneous polyherbal

preparations (n=22) were collected in polythene bags that are used to dispense products to customers by the herbal medicine practitioners. All samples were labeled and immediately transported to the Faculty of Health Science Laboratory of the Nnamdi Azikiwe University, Okofia, Nnewi.

Estimation of total viable count of bacteria

A tenfold dilution of each sample of polyherbal was achieved by adding 1ml of each sample to 9ml of sterile normal saline in the first test tube of a row of 10 sterile tubes. One (1) ml from the first tubes on each row was then transferred to the 2nd test tube after proper mixing continuing up to the 10th tube where one (1) ml of the mixture was discarded to achieve a $1/10^1$ to $1/10^{10}$ dilutions. One (1) ml of the dilution from each test tube was then transferred into a sterile Petri dish and molten nutrient agar was added, the constituents were well mixed and incubated aerobically at 37°C for 24 hours. The number of colonies on each plate was counted and the mean for each sample was established and recorded as the mean colony forming units (CFU) per ml.

Isolation and identification of bacterial contaminants in the polyherbal preparations.

The isolation and identification of the bacterial agents was done by culture on two commercial chromogenic media; CHROMagar™ Orientation and HARDYCHROM™ SS NOPRO agar, which have been validated to have positive and negative predictive values of 99.3% and 100% respectively for the isolation and identification of the bacterial organisms such as *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus* sp, *Salmonella* sp, *Citrobacter* sp, *Serratia marcescens*, *Providencia* sp, *Acinetobacter* sp, and

Pseudomonas aeruginosa which are common contaminants in polyherbal preparations (1,5). The media were prepared according to the manufacturer's instructions.

Briefly, labeled individual samples of both the packaged and unpackaged herbs were well mixed to ensure complete homogenization before culture. A loopful of each of the samples was streaked on the prepared agar plates using sterile wire loop. Incubation was done aerobically at 37°C for 24 hours. A sterile non-inoculated plate was also placed in the incubator for quality control during incubation (3). Plates were read after 24 hours and bacteria were identified by their peculiar and different colony colours and chromogenic attributes using the colour charts as a guide (7), in determining the bacterial isolates (1). The identification parameters of bacterial colonies on the media based on colour are; *Klebsiella* sp colonies appeared metallic blue, *Enterococcus faecalis* colonies as red, *Proteus mirabilis* colonies as clear and brown halo, and *Salmonella* spp as pink.

Analysis of data

The data were presented in frequency tables and statistical analysis performed with IBM SPSS 20.0 version. Chi square test was used to measure association of bacterial contamination with packed and unpackaged polyherbals, and *p* value less than 0.05 was considered to be statistically significant.

Results:

The frequency of distribution of bacterial contaminants in the packaged and unpackaged polyherbal samples is shown in Table 1.

Bacterial contaminants were isolated from 9 of 22 (40.9%) packaged polyherbal samples while 13 of 22 (59.1%) samples were bacteriologically sterile. For the unpackaged polyherbals, bacterial contaminants were isolated in 18/22 (81.8%) samples while 4/22 (18.2%) were bacteriologically sterile (OR 0.1538, *p* =0.0122). Of the 9 positive samples in the packaged polyherbals, a total of 18 bacterial isolates were recovered, *E. faecalis* was the most frequent with 33.3% (6/18), followed by *S. aureus* 16.7%, *E. coli* 16.7%, *Proteus* sp 16.7%, *K. pneumoniae* 11.1% and *Salmonella* sp 5.6%. Of the 18 positive samples in the unpackaged polyherbals, a total of 28 bacterial isolates were recovered, *S. aureus* was the most frequent with 25% (7/28), followed by *K. pneumoniae* 17.9%, *E. coli* 17.9%, *Proteus* sp 17.9%, *E. faecalis* 10.7% and *Salmonella* sp 10.7% (Table 1).

Table 2 shows the distribution of the polyherbal products from the 16 herbal shops and trade-medical hawkers, and the bacteria isolates recovered from those positive. Table 3 shows the total viability count (TVC) for each of the packaged herbal product that cultured positive for bacteria with a mean TVC of 1.48×10^6 CFU/ml, while Table 4 shows the TVC for each of the unpackaged herbal product that cultured positive for bacteria, with a mean TVC of 1.85×10^6 CFU/ml.

Discussion:

Data obtained from this study showed that bacterial agents such as *K. pneumoniae*, *E. faecalis*, *S. aureus*, *E. coli*, *P. mirabilis* and *Salmonella* sp were isolated at varying frequencies in both groups of polyherbals. These

Table 1: Frequency distribution of bacterial isolates in packaged and unpackaged polyherbal samples

No of polyherbals/ bacterial isolates	Packaged (%) (n=22)	Unpackaged (%) (n=22)	χ^2	<i>P</i>
No bacteria isolate	13 (59.1)	4 (18.8)	7.8	0.01*
No positive for bacterial isolate	9 (40.9)	18 (81.2)		
<i>Klebsiella pneumoniae</i>	2 (11.1)	5 (17.9)		
<i>Enterococcus faecalis</i>	6 (33.3)	3 (10.7)		
<i>Staphylococcus aureus</i>	3 (16.7)	7 (25)		
<i>Escherichia coli</i>	3 (16.7)	5 (17.9)		
<i>Proteus</i> sp	3 (16.7)	5 (17.9)		
<i>Salmonella</i> sp	1 (5.6)	3 (10.7)		
Total isolates	18 (100)	28 (100)		

χ^2 = Chi square; *statistically significant

Table 2: Distribution of bacterial contaminants in polyherbals purchased at the herbal shops

Herbal shops	Polyherbals	Bacterial isolates
Dan-Iyke	Katoka, Ruzu bitters, Yoyo bitters	No growth
Blessed Mother	Blood purifier, Super bitters	No growth
Dr Chiagozie	Nando mixture, Super bitters, Mako cleanser, Super 7, Ruzu bitters	<i>Enterococcus faecalis</i>
Panx	Goko cleanser, dukun care, Dr Igah cleanser	<i>E. coli</i> , <i>Klebsiella</i> sp, <i>E. faecalis</i> , <i>S. aureus</i>
Fesco	Deep root, Bitterkinga	No growth
Dr Agnes	J.M.I herbal, Museya, Jalin herbal	<i>E. faecalis</i> , <i>Klebsiella</i> sp, <i>E. coli</i> , <i>Proteus</i> sp
Eze	Eze herbal mixture, new beta cleanser	<i>E. coli</i> , <i>Proteus</i> sp
Dr Benbella	Weifa body defense	No growth
Baba Oyo	Olori herbal mixture	<i>E. faecalis</i> , <i>S. aureus</i> , <i>Proteus</i> sp
Yemi	Anti-pile, Anti-diabetic, fibroid	<i>E. coli</i> , <i>Klebsiella</i> sp, <i>E. faecalis</i> , <i>Salmonella</i> sp
Dan Obitube	Convulsion formula, general well-being, blood booster, energy booster	<i>E. coli</i> , <i>E. faecalis</i>
Titi	Fertility preparation, Laxative preparation	<i>S. aureus</i> , <i>Proteus</i> sp
Barakat	Anti-ulcer, STI preparation, Abdominal preparation	<i>S. aureus</i> , <i>Proteus</i> sp
Laide	Skin infection, menstruation prep	<i>Proteus</i> sp
Baba Osun	Male fertility, sexual health, anti-pile, back pain	<i>E. coli</i> , <i>S. aureus</i> , <i>Klebsiella</i> sp, <i>Salmonella</i> sp
Sunny	Anti-gonorrhoea, STD preparation, anti-malaria	<i>E. coli</i> , <i>Klebsiella</i> sp

Table 3: Total Viable Counts in packaged polyherbal samples and their bacteriological safety

Packaged polyherbals	Total Viable Count (CFU/ml)	Bacteriological Safety Level (10 ⁵)
Deep Root	No Isolate	Safe
Blood purifier	No Isolate	Safe
Jalin herbal mixture	1.5x10 ⁶	Unsafe
J.M.I herbal mixture	3.6x10 ⁶	Unsafe
Mako Cleanser	No Isolate	Safe
Super 7	1.4x10 ⁶	Unsafe
Museya bitters	No Isolate	Safe
Dr Igah Bitter cleanser	0.7x10 ⁶	Unsafe
Goko Cleanser	No Isolate	Safe
New Beta cleanser	No Isolate	Safe
Infection destroyer	1.3x10 ⁶	Unsafe
Dr sunny Gonorrhoea herbal	1.8x10 ⁶	Unsafe
Dukun Care	1.7x10 ⁶	Unsafe
Katoka Mixture	No Isolate	Safe
Eze herbal	3.2x10 ⁶	Unsafe
Dr Nando	No Isolate	Safe
Super bitters	No Isolate	Safe
Ruzu bitters	No Isolate	Safe
Weifa body defense	No Isolate	Safe
Bitterkinga	No Isolate	Safe
Yoyo bitters	No Isolate	Safe
Mean Total Viable Count (TVC) = 1.4845±122.17x10 ⁶		

Table 4: Total viable counts in unpackaged polyherbal samples and their bacteriological safety

Unpackaged Polyherbals	Total Viable Counts (CFU/ml)	Bacteriological safety Level 10 ⁵
Anti-malaria preparation	2.4x 10 ⁶	Unsafe
Convulsion formula	3.3x10 ⁶	Unsafe
General Well-being formula	No Isolate	Safe
Anti-Pile preparation	2.3x10 ⁶	Unsafe
Anti-Diabetic preparation	No Isolate	Safe
Fertility preparation	2.1x10 ⁶	Unsafe
Blood booster	1.9x10 ⁶	Unsafe
Back pain preparation	3.1x10 ⁶	Unsafe
Laxative preparation	8.9x10 ⁶	Unsafe
General well-being	1.8x10 ⁶	Unsafe
Anti-Gonorrhoea preparation	2.9x10 ⁶	Unsafe
Anti-ulcer preparation	1.6x 10 ⁶	Unsafe
Anti-malaria preparation	2.0x 10 ⁶	Unsafe
Male fertility preparation	1.8x10 ⁶	Unsafe
Sexual health preparation	3.7x10 ⁶	Unsafe
General wellness	3.0x10 ⁶	Unsafe
STI preparation	3.2x10 ⁶	Unsafe
Abdominal disturbance preparation	No Isolate	Safe
Skin infection preparation	No Isolate	Safe
Fibroid preparation	1.2x10 ⁶	Unsafe
Menstruation preparation	1.9x10 ⁶	Unsafe
Energy booster	1.7x10 ⁶	Unsafe
Mean Total Viable Count (TVC) = 1.856+113.27x10 ⁶		

results are similar with the findings of Esimone et al., (4) and Tاتفeng et al., (9), both of whom detected bacterial agents at varying frequencies in herbals and polyherbals samples in Nigeria. The predominance of *E. faecalis* and *S. aureus* in the packaged and unpackaged herbals respectively is in consonance with the study by Esimone et al., (4) who investigated the microbiological quality of liquid herbal preparations in south-eastern Nigeria and isolated arrays of microbial contaminants including *S. aureus* and *E. faecalis* as the most predominant bacterial contaminants of herbal medicines.

The higher frequency of contamination observed in the unpackaged group of polyherbals (81.8%) and higher median total viable count (TVC) compared to those of the packaged group may be attributable to contamination due to lack of standardization and quality control, poor personnel hygiene and handling, and use of contaminated water and raw materials (4). These findings could also be due to the fact that the packaged polyherbal products are comparatively better regulated by government agencies such as the National Agency for Food and Drug Administration and Control (NAFDAC) and the State Ministries of Health who usually mandate the manufacturers of packaged polyherbals to adopt some level of good manufacturing procedure, safe handling measures during production and quality control (6).

This study shows that many polyherbal products sold in Nnewi are potentially contaminated by bacterial agents, some of which are potential pathogens of man. It is imperative that herbal medicine practitioners be enlightened on hygienic ways of preventing microbial contamination during polyherbal production.

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**Case Report****Open Access****Hookworm presenting as acute febrile illness and surgical abdomen***^{1,2}Efunshile, A. M., ^{1,2}Ojide, C. K., ¹Nwangwu, C. C., ²Emelobe, G., and ³Akpa, C.¹Department of Medical Microbiology, Ebonyi State University, Abakaliki, Nigeria²Federal Teaching Hospital, Abakaliki, Nigeria³Department of Haematology, Federal Teaching Hospital, Abakaliki, Nigeria*Correspondence to: drefunshile@yahoo.com**Abstract:**

Hookworm is estimated to infect about 500 million people worldwide. It is usually associated with diarrhoea, abdominal pain and iron deficiency anaemia, but diagnosis can be missed because it sometimes presents unusually or mimics other diseases. We present in this report the case of an 18-year-old asthmatic medical student who was referred to a general surgeon on account of abdominal pain of 8 days duration. His problem started initially with fever, chills and rigor which was managed as malaria but no improvement. The diagnosis shifted from enteric fever to peptic ulcer disease, and his worsening condition was later reviewed as acute abdomen. Abdominal X-ray and ultrasound were normal. Stool microscopy showed numerous ova of hookworm. He was then treated with albendazole leading to full recovery on subsequent follow up. Hookworm disease can mimic a lot of other clinical conditions. A careful review of literature is a reminder that the clinical presentation of hookworm can be diverse and misleading. The current report emphasizes the fact that hookworm should always be considered a possible differential in endemic regions when clinicians are confronted with acute non-malaria febrile illnesses or cases of acute surgical abdomen.

Keywords: acute abdomen, fever, hookworm.

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Ankylostomes se présentant comme une maladie fébrile aiguë et un abdomen chirurgical*^{1,2}Efunshile, A. M., ^{1,2}Ojide, C. K., ¹Nwangwu, C. C., ²Emelobe, G., et ³Akpa, C.¹Département de microbiologie médicale, Université d'État Ebonyi, Abakaliki, Nigéria²Hôpital universitaire fédéral, Abakaliki, Nigéria³Département d'hématologie, Hôpital fédéral d'enseignement, Abakaliki, Nigéria*Correspondance à: drefunshile@yahoo.com**Abstrait:**

On estime que l'ankylostome infecte environ 500 millions de personnes dans le monde. Il est généralement associé à la diarrhée, à des douleurs abdominales et à une anémie ferriprive, mais le diagnostic peut être manqué car il présente parfois des anomalies ou imite d'autres maladies. Nous présentons dans ce rapport le cas d'un étudiant en médecine asthmatique de 18 ans qui a été référé à un chirurgien général en raison de douleurs abdominales d'une durée de 8 jours. Son problème a commencé initialement par de la fièvre, des frissons et une rigueur qui ont été gérés comme le paludisme mais sans amélioration. Le diagnostic est passé de la fièvre entérique à l'ulcère gastro-duodénal et son aggravation de la condition a ensuite été examinée en tant qu'abdomen aigu. La radiographie abdominale et l'échographie étaient normales. La microscopie des selles a montré de nombreux ovules d'ankylostome. Il a ensuite été traité avec de l'albendazole, ce qui a permis un rétablissement complet lors du suivi ultérieur. L'ankylostomiase peut imiter de nombreuses autres conditions cliniques. Un examen attentif de la littérature rappelle que la présentation clinique de l'ankylostome peut être diverse et trompeuse. Le présent rapport souligne le fait que

l'ankylostome doit toujours être considéré comme un différentiel possible dans les régions endémiques lorsque les cliniciens sont confrontés à des maladies fébriles aiguës non paludiques ou à des cas d'abdomen chirurgical aigu.

Mots-clés: abdomen aigu, fièvre, ankylostome

Introduction:

Hookworm is one of the soil transmitted helminths (STHs) which collectively constitutes a major neglected tropical disease (NTD). About 5.1 billion people are at risk of hookworm infection while an estimate of 500 million is infected worldwide (1). *Necator americanus* and *Ancylostoma duodenale* are the usual human species with global distribution and are mostly associated with low socio-economic status. Unlike the epidemiology of other STHs in which prevalence and intensity reduce with age, adults suffer higher intensity of hookworm infection when compared with young children (2,3). Therefore, mass drug administration (MDA) programs in school aged children seems to have less impact on the prevalence of hookworm, partly because adults are usually exempted (1,4).

Risk factors for hookworm infection include walking bare footed, absence of household latrine and low socio-economic status. Initial skin penetration by the larva results in "ground itch" (2) while subsequent pulmonary migration results in pneumonitis accompanied by cough, sore throat and fever. Epigastric pain may result from the arrival of the larva in the intestine and subsequent development into adult (5). Light infection may be asymptomatic, but clinical features of heavy infection include abdominal pain, diarrhea, reduced appetite, weight loss, fatigue and anaemia. Hookworm rarely causes acute illness or death but rather associated with chronic diseases such as poor growth and impaired cognitive development. Heavy infection may result in hypoproteinemia as a result of plasma protein ingestion by adult worms, whereby patients may present with edema or anasarca (6).

Unusual clinical presentations have been reported to cause missed diagnosis and misdiagnosis of hookworm infections. This helminth was once found to be responsible for chronic fever of 3 months duration in a 17-year-old Indian male (7). There was also a report of clandestine presentation as total hyphema with secondary glaucoma after an adult hookworm infected the anterior eye chamber of a 70-year-old man in India (8). *Ancylostoma caninum* was also found to be associated with unexplained abdominal pain in Australia, while dog ownership was found to be a risk factor (9). Case reports from the United Kingdom previously

showed that occultic hookworm infection was associated with acute watery diarrhoea in returned traveler without demonstrable ova and parasites in stool (10).

The diagnosis is traditionally by microscopy for ova in stool. Albendazole and mebendazole are the first line drugs of choice. Hookworm is controlled by mass drug administration (MDA) supported by health education, improved sanitation and hygiene.

Case presentation:

This was a case of an 18-year-old medical student who was referred to a general surgeon for evaluation on account of abdominal pain of 8 days duration. His problem started initially with fever associated with rigor, chills and passage of loose stool for which malaria was suspected. He was treated with artemisinin-based combination therapy but to no avail. Enteric fever was later suspected, and he was commenced on empirical antibiotics pending laboratory investigations. The abdominal pain became more severe and aggravated by eating. Clinical diagnosis of acute abdomen, with suspicion of acute appendicitis was then concluded. Analgesics, metronidazole, and amoxicillin-clavulanate were administered.

Full blood count and peripheral blood film results were normal. Abdominal X-Ray and ultrasound findings were also unremarkable. His stool was semi-solid, not dark in color and no trace of blood. The abdominal pain eventually shifted to the epigastric region which again shifted the clinical impression to peptic ulcer disease. Review of his past medical history only showed that he was asthmatic and on salbutamol inhaler for the past 10 years. On presentation, he was emotionally disturbed, otherwise healthy-looking young man, well nourished, not dehydrated and not pale. The abdomen was flat moves with respiration; tenderness was noted at the epigastric, left and right iliac regions but there was no organomegaly.

Stool immunochromatographic antigen test for *Helicobacter pylori* was negative while stool occult blood was positive. The latter finding prompted the decision for stool microscopy which showed heavy concentration of ova of hookworm. The patient was then treated with albendazole 400mg daily for 3 days. The fever and abdominal pain resolved on the second day

of albendazole administration and leading to full recovery on subsequent follow up.

Discussion:

The presence of fever in this case may be due to migration of L3 larval stage through the patient's lungs. This migration might also have been associated with pneumonitis, but the presence of cough was likely masked by the fact that the patient was asthmatic, which further enhance the misdiagnosis. The severity of the abdominal pain coupled with the absence of malnutrition, anaemia and eosinophilia that is characteristically seen in helminthic infection, probably biased the opinion of the surgeon away from hookworm as a possible diagnosis. This was similar to another report of hookworm case in the USA which presented as acute surgical abdomen, only to be confirmed as hookworm infection after emergency exploratory laparotomy was carried out (11).

Conclusion:

In conclusion, a careful review of literature is a reminder of the fact that the clinical presentation of hookworm could be very diverse and misleading. Diagnosis can be further complicated because it can mimic a lot of other disease conditions, hence clinicians need to stay updated about these possibilities. The current report emphasizes the fact that hookworm should always be considered a possible differential in endemic regions when clinicians are confronted with acute non-malaria febrile illnesses or cases of acute surgical abdomen.

Conflict of interest:

No conflict of interest is declared.

Ethical consideration:

Informed consent was duly obtained from the patient

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