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Copyright AJCEM 2021: <https://dx.doi.org/10.4314/ajcem.v22i1.1>**Viewpoint****Open Access****Africa's COVID-19 story: cheap innovation technology and climate protective effect to her rescue?**

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\*Correspondence to: [dapoadesokan@gmail.com](mailto:dapoadesokan@gmail.com); [Ade@precisemed.co.uk](mailto:Ade@precisemed.co.uk)**Abstract:**

As the COVID-19 pandemic sweeps the globe, causing tens of thousands of deaths in most Western countries with economies round the world in turmoil, Africa has so far been largely spared the kind of impact that has thrown the United States, South America and Europe into crisis. Most African countries remain seriously unprepared to handle the pandemic of the nature the Western world is dealing with; Africa, from Mali to Ethiopia to Libya and down to South Africa, have insufficient ventilators or intensive care beds to cope with COVID-19 should it strike with ferocity as it is doing in the Western world. As COVID-19 reaches the shores of Africa, despite poor health facilities, poor living conditions and inadequate availability of clean water across the continent, Africans are still putting up a fight taking COVID-19 head on with use of cheap technology, and help from the continent's protective climate. However, Africa cannot afford to be complacent. African countries must continue to adopt strict social distancing measures, educate their people on the importance of intake of regular vitamin D, good exercising habit, good sleep pattern, adequate hand hygiene measures, as well as strictly enforcing the "test, trace and isolate" model to the letter for the continent to take on the fight head on and wage a proper war against COVID-19.

**Keywords:** SARS-COV-2; COVID-19; innovation; technology; climate; Africa

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**L'histoire du COVID-19 en Afrique: une technologie d'innovation bon marché et un effet protecteur du climat à sa rescousse?**

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\*Correspondance à: [dapoadesokan@gmail.com](mailto:dapoadesokan@gmail.com); [Ade@precisemed.co.uk](mailto:Ade@precisemed.co.uk)**Abstrait:**

Alors que la pandémie du COVID-19 balaie le globe, causant des dizaines de milliers de morts dans la plupart des pays occidentaux avec des économies du monde entier en crise, l'Afrique a jusqu'à présent été largement épargnée par le type d'impact qui a jeté les États-Unis, l'Amérique du Sud et l'Europe en crise. La plupart des pays africains ne sont pas vraiment préparés à faire face à la pandémie de la nature à laquelle le monde occidental est confronté; L'Afrique, du Mali à l'Éthiopie en passant par la Libye et jusqu'en Afrique du Sud, ne dispose pas de ventilateurs ou de lits de soins intensifs insuffisants pour faire face au COVID-19 s'il frappe avec férocité comme dans le monde occidental. Alors que le COVID-19 atteint les rives de l'Afrique, malgré des installations de santé médiocres, des conditions de vie médiocres et une disponibilité insuffisante d'eau potable à travers le continent, les Africains continuent de se battre en prenant le COVID-19 de front avec l'utilisation d'une technologie bon marché et l'aide de le climat protecteur du continent. Cependant, l'Afrique ne peut pas se permettre d'être complaisante. Les pays africains doivent continuer à adopter des mesures de distanciation sociale strictes, éduquer leur population sur l'importance de l'apport régulier de vitamine D, de bonnes habitudes d'exercice, un bon sommeil, des mesures d'hygiène des mains adéquates, ainsi que l'application stricte du «test, traçage et isolement». modèle à la lettre pour que le continent se batte de front et mène une véritable guerre contre le COVID-19.

**Mots clés:** SRAS-COV-2; COVID-19; innovation; La technologie; climat; Afrique

## Introduction:

Coronavirus is a large family of respiratory viruses; pathogenic examples are the severe acute respiratory syndrome-coronavirus (SARS-COV), the Middle East respiratory syndrome-coronavirus (MERS-COV), SARS-COV2, and other coronaviruses that cause common cold. They are found in animal hosts such as camels, cattle, bats and other animals. Coronaviruses have caused two large-scale pandemics in the past two decades; SARS and MERS (1,2). The current pandemic, COVID-19 caused by SARS-COV-2, was first reported in Wuhan China on December 31<sup>st</sup>, 2019 and soon became a global disease of immense significance, due to global travel. It is transmitted mainly through inhalation of respiratory droplets, but other means of transmission include contact with infected persons and surfaces, as well as inhalation of SARS-COV-2 infected aerosols.

Since World War II, the world has not witnessed anything of such magnitude as COVID-19. The pandemic so far has struck and stretched the heart of the western world beyond many of its individual nations' health system capacities. Therefore, it is fair for the rest of world to fear for Africa, the continent home to 1.2 billion people, without sufficient ventilators, personal protective equipment (PPE), testing capacity, and intensive care unit beds. Most African countries remain seriously unprepared to handle the pandemic of the nature the western world is dealing with. Compared to the United States which has 34 beds for every 100,000 persons (3), African countries have insufficient ventilators or intensive care beds to cope with COVID-19 should it strike with the ferocity observed in the Western World.

In most parts of Africa, people live in overcrowded conditions making social distancing, a critical prevention COVID-19 strategy, a practical impossibility. Millions of Africans live without access to clean running water, which makes frequent hand washing also a difficult task (4). Most African countries were already or suffering from the overburdening effect of tuberculosis, HIV/ AIDS, and non-communicable diseases, and infectious diseases such as cholera, measles, and malaria. Adding COVID-19 to the mix could draw the analogy of the straw that broke the camel's back. While under pressure, intensive care units in Europe and the US were complaining of lack of adequate ventilators and PPEs in the early days of the COVID-19 outbreak, most African countries could only rely on hand hygiene, social distancing measures, and isolation centres to fight the deadly scourge of COVID-19.

The COVID-19 pattern globally, is that 80% of those infected will not require hospitalization,

that is either because they end up as asymptomatic carriers or go on to be mildly symptomatic, and about 20% could become significantly ill with COVID-19. Most of the latter will need oxygen treatment, and about a third of this hospitalized population will result in critical cases requiring continuous positive airway pressure (CPAP) or mechanical ventilation (5). Without such advanced care for this critically ill group of people, death by complications of acute respiratory distress syndrome (ARDS) and widespread micro-thrombosis, which is the hallmark of critical COVID-19, is all but inevitable.

In Western countries, hospital availability of oxygen to treat patients is taken for granted. In Africa, few countries have hospitals where oxygen is available, and where they do exist, oxygen concentrators demand stable electricity supplies which are not always available. In an analytical study of the critical care capacity across 54 African countries (6), there was an average of 3.10 ICU beds and 0.97 ventilators per 100,000 people. The average number of ICU beds per 100,000 people ranged from 0.53 in low-income countries to 8.59 in upper-middle income countries, the highest being 33.07 in Seychelles, the only high-income country included in the analysis (6). The average number of ventilators per 100,000 people ranged from 0.14 in low-income countries to 2.49 in upper-middle-income countries. The average number of ICU beds was lowest in West Africa with only 1.10 ICU bed per 100,000 people, and the average number of ventilators was lowest in East Africa with only 0.23 ventilators per 100,000 people. There was an average of 2.42 total (physician and non-physician) anaesthesia providers per 100,000 people ranging from 1.24 and 0.66 in low-income countries and the Middle African region respectively, to 6.91 and 6.64 per 100,000 people in the upper-middle-income countries and the North Africa region, respectively.

As the COVID-19 pandemic sweeps the globe, causing tens of thousands of deaths in Western countries and throwing economies round the world into turmoil, Africa has so far been largely spared the kind of impact that has thrown the United States, South America and Europe into crisis. As of mid-April, there were about 14,000 confirmed cases on the African continent, as compared with 160,000 in Italy and more than 560,000 in the United States (7,8). Despite the slow arrival of the pandemic in Africa, the numbers are slowly rising, implying the 1.2 billion people living in Africa are at tremendous risk.

It is fair to assume that one of the reasons African countries are reporting low rates of infection is due to its limited testing capacity. As at April 9, 2020, Africa CDC reported that Nigeria with a population of 200

million people had only conducted 6,000 tests and Ethiopia with a population of about 100 million people had conducted about 5,000 tests (9). While this is true for some countries, the likes of Senegal, Ghana, South Africa, Mauritius, and others have ramped up their testing capability. However, African countries continue to record low mortality rates of COVID-19 which can be gauged from level of hospitalizations and number of deaths with history of respiratory symptoms, should the actual tests for COVID-19 not be available. Therefore, this viewpoint is an attempt to hypothesize the reasons for the low COVID-19 mortality rates in Africa.

## **Cheap COVID-19 technology**

Confronting epidemics is not new to Africans, and their previous challenging, but successful experiences may prove to be pivotal in the fight against COVID-19. For generations, Africans have been responding to infectious diseases, both governments and communities realise the need for rapid, proactive measures to save lives in the face of scarce advanced health facilities and resources. Over the years, Africans have devised their own traditional treatment modalities and technologies, through simple and low-cost ways of fighting different infectious diseases that have come their way in the past. Moreover, a substantial number of countries in Africa have benefited from previous global initiatives to strengthen health systems to address HIV, malaria, tuberculosis, and Ebola (10). In addition, the Africa CDC has accelerated its work to enhance diagnostic and surveillance capacity on the continent since the start of the outbreak of the COVID-19, and is putting together an effort to distribute one million test kits across Africa (9).

Senegal is the role model of the continent, where local experts are led by Amadou Sall, the Director of WHO Collaborating centre for Arboviruses and viral haemorrhagic fever at the Institut Pasteur de Dakar. His team is leading the way in curbing the COVID-19 pandemic by providing testing for all its citizens. The West African nation is engaging in the "test, trace and isolate" model to good effect, building on its experience in fighting HIV/AIDS and Ebola. The Institut Pasteur is creating a 3D printed \$60 ventilator and \$1 testing kit so cost-effective and of high quality that the British Biotech company, Mologic, is engaging in collaboration with Institut Pasteur's Biotech Dia Tropix to make 10 minutes test kits (11). The institute plans to make 2 to 4 million diagnostic test kits available soon in the fight against COVID-19 pandemic. Senegal has put resources in place to share its other strategies with other countries on the continent.

Immediately after the news of the outbreak was confirmed in January, Rwanda, another shining light on the continent for the last 20 years, after putting the horrors of genocide behind it, trained about 500 health workers to deal with a potential national epidemic, putting strict social distancing guidance with a focused and result-oriented leadership from President Paul Kagame. Rwanda has deployed five state-of-the-art humanoid robots to aid in fight against COVID-19. Health Ministry officials said that the robots could deliver food and medication and screen the temperatures of 50 to 150 people per minute. The Rwandans gave their COVID-19 robots local names; Mwiza, Ikizere, Akazuba, Urumuri and Ngabo.

Mauritius, the tiny African holiday island resort, enforced an early lockdown and rolled out mass testing capable of testing 10% of its population with 100,000 tests carried out within two weeks of the outbreak. Its health-care facilities, which boast of 3.4 hospital beds per 1,000 people (12) competes favourably with the giants of the Western world. Its health workers are well prepared should the pandemic take a turn for the worse.

In Nigeria, the advent of COVID-19 has bred innovation in terms of development of ventilators by the likes of the National Defence Academy (NDA) and several Universities, as well as factories making PPEs in form of face shields, face masks and gowns. Drive-through COVID-19 testing and mobile disinfectant units are also being developed and deployed around the country. People who are suspected of showing symptoms can have their symptoms registered online and are screened to ascertain whether they qualify for a test; and can then drive through a testing centre and receive their results electronically.

In Kenya, at the large regional hospitals, teams of health workers have set up tents to provide information on COVID-19, take temperatures readings and log the travel histories of the local residents before they enter the hospitals. The deployment of these cheap and effective technologies across Africa is a welcome development and should be further supported by individual governments in the fight against the pandemic.

## **Africa's climate protective effect**

The Spanish flu of 1918 affected 500 million people and killed about 50 million worldwide (~10% mortality rate), including an estimated 675,000 people in the United States (13,14). Italy's COVID-19 mortality rate as at 4<sup>th</sup> of April 2020 stood at 17% with 15,362 deaths out of 88,274 recorded infections (15), making Italy the nation with the highest death rate worldwide, far worse death rate than the Spanish flu's over 100 years ago.

Africa, home to nearly 1.2 billion people, continues to puzzle scientists why it has the least infection rates globally. Majority of cases in West Africa are index cases brought in by travellers from Europe, US, and South East Asia. South Africa has 1,000 cases with 9 deaths as at 4<sup>th</sup> of April, 2020; and Nigeria has 214 cases and 4 deaths (16), which implies that as at 4<sup>th</sup> of April, 2020, Africa has hardly had more than 100 deaths, neither has Africa recorded more than 100,000 cases (16). All these may indicate there could be a reason for this negligible mortality rate compared to the rest of the world. The hypothesis here is that to be highly transmissible, SARS-COV-2 virus requires temperatures between 2-13°C for infectivity and transmission judging by the finding from the Chinese study (17). The Chinese study found that in areas of average temperature of 18°C, infection rates are less than 5%. Also, areas in China with hot and humid weather had slower waves of transmission of COVID-19. Most regions of Africa have temperatures averaging between 25°C - 40°C as at April 2020, therefore the theory is that hot, humid climate slows down the community spread of SARS-COV-2, hence the low infection and mortality rates recorded in Africa thus far.

Humidity is postulated to affect the transmission of Influenza outbreak in a similar manner to COVID-19. Small mucus droplets spread in the air in hot, humid conditions, and loses its infectivity because the particles lose their structural integrity in such conditions. The fact that respiratory droplets become less virulent in humid and tropical weather, might account for the low mortality rates seen in Africa. To buttress this low virulence, low mortality climate theory, hotter and humid areas of China like Beijing and Shanghai had lower infection rates, and lower mortality rates compared to more temperate regions like Wuhan Hubei province (17). The Chinese study revealed that there were in total 24,139 confirmed cases in China and 26 overseas countries, of which, 16,480 cases (68.01%) were from Hubei Province (17). The number of cumulative total confirmed cases (lgN) rose as the average temperature went up to a peak of 8.72°C and then slowly declined. The apexes of the minimum and maximum temperatures were 6.70°C and 12.42°C respectively, and the curves shared similar shapes. Under the circumstance of lower temperature, every 1°C decrease in average, minimum and maximum temperatures led to an increase of the cumulative number of cases by 0.83, 0.82 and 0.83 respectively.

In the single-factor model of the higher-temperature group, every 1°C increase in the minimum temperature led to a decrease

of the cumulative number of cases by 0.86 (17). The downside to this theory is the fact that Africa like the rest of the world has 2 seasons; summer and winter. This however uniquely occurs at the same time in different parts of the continent simultaneously. As of mid-April 2020, there were about 14,000 confirmed cases on the African continent, compared to figures by 16<sup>th</sup> of August 2020, where Africa's COVID-19 cases had passed the 1.1 million mark (18). Despite the slow arrival of COVID-19, the numbers have risen steadily when the figures obtained in April are compared to that of August, implying the 1.2 billion people living in Africa are at tremendous risk. The reason for this is likely due to the fact that, when COVID-19 emerged, most of Africa in the tropics had their dry, hot season ongoing, compared to April to October when the raining, colder and wetter season starts and encouraging more COVID-19 infections owing to plummeting temperature and humidity as earlier hypothesised.

In Nigeria for example, the dry season typically runs from November to March, with temperatures that average about 37°C during the day, while the wet season typically runs from April to October. During this time, the southern part of the country alone can receive more than 150 inches of rainfall, which invariably cause drop in temperature. Thus wetter, colder climate encourages spread of respiratory viruses like SARS-COV-2 and Influenza as earlier highlighted. In contrast, on the same continent, the climate of South Africa is uniquely different from most parts of Africa because it lies between two oceans; Atlantic and Indian, in 22°S and 35°S latitude in the Southern Hemisphere. This confers upon the country a subtropical climate with lower temperatures even in the "summer" dry season compared to the rest of Africa with tropical climate all year round.

Interestingly, to buttress this different climate conferring different COVID-19 rates theory, as of August 16, 2020 according to Johns Hopkins University data (18), worldwide proven infection rate stood at 21,593,607 with 773,685 deaths and 13,594,900 recoveries. During the same period, South Africa had recorded a total of 587,345 infections with 11,839 deaths compared to Nigeria's 49,068 infections and 975 deaths. The reason for this very significant disparity in number of infections and deaths is likely due to the fact that while COVID-19 started during Nigeria's dry, hot season, which is in contrast to South Africa, where it started during winter in its subtropical climate. Thus, South Africa's colder climate like the rest of temperate world as highlighted earlier in this viewpoint could be argued as facilitating more infections and



invariably higher death rates compared to Nigeria's or the rest of Africa with tropical climate all year round.

## Discussion

As the COVID-19 pandemic sweeps across the globe, causing tens of thousands of deaths in most Western countries and massive economic disruption, Africa has so far been largely being spared the kind of impact that has thrown the United States, and Europe into crisis. When it reached the shores of Africa, despite poor health facilities, poor living conditions and unavailability of clean water across the continent, Africans' still put up a fight to take COVID-19 head on with use of cheap technology, with further help from the continent's protective climate. The likes of Senegal's' flagship \$1 COVID-19 test kit and \$60 ventilator are initiatives that would make the rest of the world marvel.

Scientists across Africa have also proposed the cross immunities theory, arguing that malaria and other infectious diseases endemicity in Africa might have protective effect on the low COVID-19 infection and mortality rates observed in Africa. However, the fact that Africans living in the western world who grew up in Africa exposed to these infectious diseases died from COVID-19 related causes in substantial number in the western world adds little credence to this theory.

Going forward in the face of limited resources to fight this pandemic, if Africa must wage a formidable and viable war against this "invisible enemy" COVID-19, it must adhere to excellent use of public health tools. It must also have an effective Test, Trace & Isolate model. Social distancing and hand hygiene education must become the norm and the continent must invest in local cheap PPE and diagnostics technology. Lockdowns should continue to be enforced where necessary and there should be an acute awareness of the language of the COVID-19 virus; "COVID-19 does not move, People move it, we stop moving, the virus stop moving", It is that simple.

However, all said and done, Africa cannot afford to rest on its laurels. African countries must educate its people on the importance of regular intake of Vitamin D, good exercising habit, good sleep pattern, adequate hand hygiene measures, as well as strictly enforcing the "test, trace and isolate" model to the letter. The good gesture and measures like that of President Donald Trump planning who donated to Nigeria 200 ventilators and South Africa 1000 ventilators, is highly commendable.

In the face of possible catastrophic consequences of COVID-19 should it strike

Africa as it has struck the West, the United Nation Economic Commission for Africa (UNECA) reported a worst-case scenario for Africa projecting that between 300,000 and 3.3 million people could die from COVID-19 in Africa (19). Sadly, the practice of medical tourism by African leaders and their families even for minor ailments, and their highly corrupt practices may have finally caught up with Africa with the advent of this pandemic. African leaders have misplaced their priorities over the years, by not putting to good use the vast human and natural resources at its disposal to provide adequate health care and infrastructures for Africans in Africa. Nigeria has made more than \$400 billion from crude oil sales alone since 1960. South Africa's mining wealth is worth around \$2.5 trillion, while Democratic Republic of Congo's mineral reserves is worth \$24 trillion, to mention a few. Sadly, the whole continent cannot boast of up to 2000 working ventilators worth \$15,000 a piece (\$30 million in total) to fight the scourge of COVID-19 pandemic.

On the bright side, COVID-19 might be the much-needed impetus African leaders need to wake up and get their priorities right by using the opportunity to develop our decaying and moribund health systems. Therefore, should future pandemics emerge, Africa would be in good stead to take on the fight head on and wage a proper war.

## About the principal author

Dr Adedapo Adesokan is a Physician Pharmacologist who runs a University of Strathclyde, Scotland supported start-up, PreciseMed, a pharmaceutical R&D company set up in 2017 for developing novel medical devices and pharmaceutical products for unmet clinical needs.

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**Mini Review****Open Access****Use of dexamethasone in the management of respiratory tract infections**<sup>1</sup>\*Adegboro, B., <sup>2</sup>Abayomi, S. A., <sup>1</sup>Imran, J., and <sup>3</sup>Sanni, E. O.<sup>1</sup>Department of Medical Microbiology and Immunology, Nile University of Nigeria, Abuja, Nigeria<sup>2</sup>Department of Medical Microbiology, LAUTECH Teaching Hospital, Ogbomoso, Nigeria<sup>3</sup>Department of Haematology, Nile University of Nigeria, Abuja, Nigeria\*Correspondence to: [boazadegboro@gmail.com](mailto:boazadegboro@gmail.com)**Abstract:**

Dexamethasone is a potent synthetic member of the glucocorticoid class of corticosteroid drugs that has been useful for the management of some pathological disorders because it affects a protean number of signaling pathways. It is used as adjunct therapy in the management of sepsis, arthritis, cardiac transplant, blood, hormone/immune system disorders, allergic reaction, skin, eye conditions, cancer and other pathologic disorders and as a mainstay of therapy in autoimmune hepatitis. With the advent of COVID-19, there have been investigations of its use as anti-inflammatory agent in severely ill patients. This present review elucidates the various studies on the use of dexamethasone in the management of severe respiratory tract infections, with the ultimate aim of reducing mortality amongst severely ill patients, including COVID-19.

**Keywords:** dexamethasone; adjunctive therapy; respiratory infections; COVID-19

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**Utilisation de la dexaméthasone dans la prise en charge des infections des voies respiratoires**<sup>1</sup>\*Adegboro, B., <sup>2</sup>Abayomi, S. A., <sup>1</sup>Imran, J., et <sup>3</sup>Sanni, E. O.<sup>1</sup>Département de microbiologie médicale et d'immunologie, Université du Nil du Nigéria, Abuja, Nigéria<sup>2</sup>Département de microbiologie médicale, LAUTECH Teaching Hospital, Ogbomoso, Nigéria<sup>3</sup>Département d'hématologie, Université du Nil du Nigéria, Abuja, Nigéria\*Correspondance à: [boazadegboro@gmail.com](mailto:boazadegboro@gmail.com)**Abstrait:**

La dexaméthasone est un membre synthétique puissant de la classe des corticostéroïdes glucocorticoïdes qui a été utile pour la gestion de certains troubles pathologiques car elle affecte un nombre protéiforme de voies de signalisation. Il est utilisé comme traitement d'appoint dans la prise en charge de la septicémie, de l'arthrite, de la transplantation cardiaque, du sang, des troubles hormonaux/du système immunitaire, des réactions allergiques, des affections cutanées, oculaires, du cancer et d'autres troubles pathologiques et comme pilier du traitement de l'hépatite auto-immune. Avec l'avènement du COVID-19, des études ont été menées sur son utilisation comme agent anti-inflammatoire chez des patients gravement malades. Cette revue présente les différentes études sur l'utilisation de la dexaméthasone dans la prise en charge des infections sévères des voies respiratoires, dans le but ultime de réduire la mortalité chez les patients gravement malades, y compris le COVID-19.

**Mots clés:** dexaméthasone; thérapie d'appoint; infections respiratoires; COVID-19

## Introduction:

Dexamethasone is a potent synthetic member of the glucocorticoid class of corticosteroid drugs (1). It was found useful for managing some pathological disorders because it affects a protean number of signaling pathways. These effects may explain its therapeutic benefits when used in the management of sepsis, arthritis, cardiac transplant, blood, hormone/immune system disorders, allergic reaction, skin, eye conditions, cancer and other pathologic disorders (1). Dexamethasone is synthesized by dehydration of 16  $\beta$ -methylprednisolone acetate to give the 9,11 dehydro derivative, which is then reacted with a hydrobromite source such as basic N, bromosuccinimide to form 9 $\alpha$ -bromo 11- $\beta$  hydrin derivative, and the ring is then closed to form an epoxide. A ring opening reaction with hydrogen fluoride in tetrahydrofuran gives rise to dexamethasone (2). The adrenal glands also produce corticosteroids naturally (3). These are steroid hormones which play important physiologic roles in the body such as glucose and protein metabolisms, and suppression of immune and inflammatory processes (3).

Most children will be infected by respiratory syncytial virus (RSV) before the age of two years (3). About 1% of them will need to be admitted to the hospital while respiratory failure will occur in 5-8% of cases, necessitating mechanical ventilation (3). Aspergillosis, asthma, and allergic broncho-pulmonary pneumonia could cause reactive airway diseases, and corticosteroids could be useful in treatment of these conditions (4). Corticosteroids are also useful in the management of chronic obstructive pulmonary diseases (COPD), sarcoidosis, collagen vascular diseases, eosinophilic pneumonitis, idiopathic interstitial pneumonia and infectious disorders such as laryngo-tracheo-bronchitis (4). In addition, patients with influenza virus (H1N1 strain) and severe coronavirus infections often need mechanical ventilation, and dexamethasone could be useful in their management (4).

The efficacy of corticosteroids in the management of patients with RSV-LRTI (lower respiratory tract infection) has been studied over the years with conflicting results. While some studies found beneficial effects, many well-designed randomized control trials did not show that corticosteroids are beneficial. However, prednisolone shortened the length of hospital stay for patients on mechanical ventilation. A relevant research on influenza associated pneumonia showed that low-moderate dose of corticosteroid reduced mortality in patients

with oxygen index lower than 300 mmHg (5). The purpose of this review is to assess the use of dexamethasone in the treatment of respiratory tract infections (RTIs) with emphasis on its use for patients with COVID-19.

## Dexamethasone use in upper respiratory tract infections

The upper airway (nasopharynx, oropharynx, and laryngopharynx) conveys gases to and from the lungs, and filters, warms, and humidifies the air. The trachea and bronchi are lined by pseudo-stratified ciliated columnar epithelium which forms an active physical barrier against pathogens as an important part of the innate immunity (6). Goblet cells and mucus-producing glands are also included in this area and are responsible for producing roughly 100 ml of fluids/day in the adult, or more especially in disease state. All these provide protection against respiratory viral infections (6,7). However, despite these protective mechanisms, infection occurs by virus binding to specific receptors on epithelial cells of respiratory mucosa, thereby circumventing its removal by the mucociliary system or phagocytic cells (7).

In spite of its clinical applications for up to 70 years, the role of corticosteroid as potent anti-inflammatory drug is still controversial in many pulmonary conditions (8). The role of dexamethasone in established viral infections such as severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection (COVID-19), has been variously reported (7). Following entry into the cytoplasm of the cell by passive diffusion, glucocorticoids interact with glucocorticoid receptor (GR) to form a complex, which then moves into the nucleus where they either suppress or stimulate transcription, through a process called trans-repression or transactivation (9,10). Glucocorticoids inhibit transcription factors such as nuclear factor kappa B (NF- $\kappa$ B) which are proteins that control the rate of transcription. Production of pro-inflammatory mediators by macrophages, eosinophils, lymphocytes, mast cells, and dendritic cells are controlled by these transcription factors. Also important is the inhibitory effect on phospholipase A2 that produce various inflammatory mediators (10,11).

Glucocorticoids also inhibits the genes responsible for expression of cyclooxygenase-2, inducible nitric oxide synthase, and proinflammatory cytokines, tumor necrosis factor alpha and interleukins (9). In contrast, corticosteroids initiate upregulation of lipocortin and of annexin A1, a protein that reduces prostaglandin and leukotriene synthesis and that also inhibits cyclooxygenase-2 activity and reduces

neutrophil migration to inflammatory sites. Because corticosteroid action occurs intracellularly, the effects persist, even when detection in the plasma is absent (9,10,12). Cytokine storm has been implicated as the mainstay in the pathogenesis of COVID-19 (13). The use of glucocorticoids will therefore have a place in the management of the disease. Dexamethasone is superior and preferred among other glucocorticoids due to its longer half-life and duration of action (24–36 hours), higher anti-inflammatory activities (30 times higher than cortisol and six times higher than triamcinolone), zero salt-retaining ability and most excellent penetration of lipid barriers for topical activity (10).

#### **Dexamethasone use in lower respiratory tract infection**

Following lower respiratory tract infection by any microorganism, cytokines and other inflammatory mediators are released by alveolar macrophages, which improves opsonization of the invading pathogens and usually lead to their successful removal (14). However, profound release of these cytokines and other inflammatory mediators can be detrimental to the host leading to significant damage to the lung parenchyma (14).

In a prospective study by Meduri et al., (15) involving 27 consecutive patients with acute respiratory distress syndrome (ARDS), patients who died from ARDS had raised levels of cytokines and inflammatory mediators on the first day of their admission and throughout their period of hospitalization. The persistent inflammatory response may contribute significantly to lung injury and subsequent respiratory failure. The need to abate the excessive release of cytokine and inflammatory mediators in lung infections using dexamethasone has generated wide interest among researchers, although the routine uses of systemic corticosteroid in the management of COVID-19 pneumonia has not been recommended by the World Health Organization (16). Administration of dexamethasone leads to reduction in serum levels of cytokines and inflammatory cells, which subsequently reduce the cytokine storm to the barest minimum. Mortality amongst very ill patients with ARDS is thus greatly reduced. Dexamethasone as a glucocorticoid as well as an anti-inflammatory steroid, suppress expression of pro-inflammatory genes (17).

Corticosteroids also help reduce tissue injury and edema, and this is believed to alleviate patient's distress from the accompanying inflammatory process after tissue injury. The effects of dexamethasone use on

outcome in patients with pneumonia however remains controversial. In a prospective study by Monton et al., (18) on 27 patients with severe pneumonia on mechanical ventilation, glucocorticoid (GC), dexamethasone, alleviated patient's inflammatory response state. In this study, mortality rate in the patients who had treatment with GC was 36% (4/11), while for patients who did not receive GC, mortality rate was 67% (6/9). The survivors who received GC had reduced levels of serum TNF- $\alpha$  when compared to non survivors irrespective of their treatment with GC (18). It is good to note that despite the favorable outcome in reduction of risk of mortality reported in this study, other researchers have noticed a deleterious effect of use of corticosteroids in the treatment of lower respiratory tract infection. In the systematic review and meta-analysis involving 6548 patients, Ni et al., (19) reported that corticosteroid use could increase the death rate in patients with influenza pneumonia.

As dexamethasone suppresses immune reactions by inhibiting inflammatory responses, thus inhibiting the migration of inflammatory mediators from the circulation to tissues via inhibition of the synthesis of chemokines and cytokines (19), the fear of researchers is that modulation of immune responses caused by these corticosteroids can elongate the period of viraemia and hinder viral clearance, thereby increasing the risk of mortality (20). The report of prospective study by Li et al., (5) involving 2141 hospitalized adolescent and adult patients with influenza A (H1N1) pdm09 viral pneumonia from 407 hospitals in mainland China, shows that low mortality rate was noticed in patients with severe disease treated with low-to-moderate dose corticosteroid, while patients with influenza pneumonia who had mild disease did not benefit from corticosteroid therapy. Meijvis et al., (22) also reported a significant reduction in the period of hospital admission in 304 adult patients diagnosed with community-acquired pneumonia and treated with intravenous dexamethasone (5 mg once a day) or placebo for 4 days at two teaching hospitals in the Netherlands. However, these patients also had antibiotics included in their treatment and were non-immunocompromised (21).

In a recent large randomized controlled study, 2104 patients with COVID-19 in the United Kingdom, randomly allocated to receive dexamethasone and other usual care (which included oxygen support, mechanical ventilation/intensive care, treatment of intercurrent infections/diseases), were compared with 4321 patients concurrently allocated to usual care only without administration of dexamethasone.

Dexamethasone reduced deaths by one-third in patients receiving invasive mechanical ventilation [29.0 vs 40.7%, age-adjusted rate ratio (RR); 0.65 (confidence interval 0.51 -0.82);  $p < 0.001$ ]; by one-fifth in patients receiving oxygen without invasive mechanical ventilation [1.5 vs 25.0%, RR 0.80 (95% CI 0.70 - 0.92);  $p = 0.002$ ]; but did not reduce mortality in patients not receiving respiratory support at randomization (17.0 vs 13.2%, RR 1.22 (95% CI 0.93-1.61);  $p = 0.14$ ]. In patients hospitalized with COVID-19, dexamethasone reduced 28-day mortality among those receiving invasive mechanical ventilation or oxygen at randomization, but not among patients not receiving respiratory support (22).

## Discussion:

Dexamethasone is derived from cortisol (hydrocortisone) (2). It plays important physiologic roles in the body such as glucose and protein metabolism, and suppression of immune and inflammatory processes (3). About 1% of children with RSV infection need to be admitted to hospital while respiratory failure progresses in 5-8% of them, necessitating mechanical ventilation (3). Patients with severe RSV, influenza virus (H1N1), and coronavirus infections often need mechanical ventilation, and dexamethasone could be useful in their management (4). Relevant research has also shown that influenza associated pneumonia in patients with oxygen index lower than 300 mmHg, low to moderate dose of corticosteroids significantly reduced mortality (5). Low oxygen index is a usual finding in COVID-19 patients with moderate to severe disease who require assisted ventilation. Although the role of corticosteroids as potent anti-inflammatory drugs is still controversial in the management of many pulmonary conditions, dexamethasone has been found useful in reducing mortality in established viral infections such as COVID-19 (7,8).

Among the glucocorticoids, dexamethasone is superior and preferred due to its longer half-life and duration of action (24–36 hours), its higher anti-inflammatory activities (30 times higher than cortisol and 6 times higher than triamcinolone), zero salt-retaining ability and most excellent penetration of lipid barriers for topical activity (10). In patients hospitalized with COVID-19, dexamethasone reduced 28-day mortality among those receiving invasive mechanical ventilation or oxygen at randomization, but not among patients not receiving respiratory support (22). It could therefore be safely concluded that patients with COVID-19 experiencing cytokine storm, particularly those

on mechanical/assisted ventilation will benefit from treatment with dexamethasone.

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Copyright AJCEM 2021: <https://dx.doi.org/10.4314/ajcem.v22i1.3>**Review Article****Open Access****Enterovirus and Parechovirus meningitis in children: a review of the epidemiology, diagnostic challenges, and significance of on-site CSF virology tests in tropical paediatric patients' care**<sup>1,2</sup>Kabuga, A. I., <sup>1</sup>Nejati, A., and <sup>\*1,3</sup>Shahmahmoodi, S.<sup>1</sup>Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran<sup>2</sup>Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences, Bayero University, PMB 3011 Kano, Kano State, Nigeria<sup>3</sup>Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran\*Correspondence to: [shahmahmoodi@tums.ac.ir](mailto:shahmahmoodi@tums.ac.ir); +989121909972**Abstract:**

Enteroviruses and Parechoviruses are increasingly recognized as the cause of aseptic meningitis, especially in the paediatric age group. However, because of indistinguishable clinical features with bacterial meningitis, many clinicians cannot make a clear distinction in disease presentation, and a large number of cases go undiagnosed. Although polymerase chain reaction is the current standard diagnostic approach, it takes many hours or days to get a result and these tests are not available at primary and secondary levels of care in many resource-poor countries. Furthermore, diagnosis is often difficult in children due to nonspecific cellular and biochemical cerebrospinal fluid findings. Some affected children may develop neurologic or/and systemic complications, resulting in prolonged hospital admission, increasing the risk of avoidable deaths, and healthcare expenditures. This review focuses on epidemiology, presentation, and diagnosis of Enterovirus and Parechovirus meningitis, highlighting the challenges in diagnosis and the potential roles of on-site CSF virology tests in improving the quality of paediatric patient's care. The information provided should help early case detection, thereby ensuring avoidance of unnecessary antibiotics, minimal complications, a short period of hospital stays, and a reduction in healthcare-associated costs.

**Keywords:** Aseptic meningitis; Enterovirus; Parechovirus; Diagnostic challenge; On-site virology test; Children

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**Méningite à Entérovirus et Parechovirus chez les enfants: un examen de l'épidémiologie, des défis diagnostiques et de l'importance du tests virologique sur site du LCR dans les soins aux patients pédiatriques tropicaux**<sup>1,2</sup>Kabuga, A. I., <sup>1</sup>Nejati, A., et <sup>\*1,3</sup>Shahmahmoodi, S.<sup>1</sup>Département de virologie, École de santé publique, Université des sciences médicales de Téhéran, Téhéran, Iran<sup>2</sup>Département de microbiologie médicale et parasitologie, Faculté des sciences cliniques, Collège des sciences de la santé, Université de Bayero, PMB 3011 Kano, État de Kano, Nigéria<sup>3</sup>Centre de recherche en microbiologie alimentaire, Université des sciences médicales de Téhéran, Téhéran, Iran\*Correspondance à: [shahmahmoodi@tums.ac.ir](mailto:shahmahmoodi@tums.ac.ir); +989121909972**Abstrait:**

Les entérovirus et les parechovirus sont de plus en plus reconnus comme la cause de la méningite aseptique, en particulier dans le groupe d'âge pédiatrique. Cependant, en raison des caractéristiques cliniques indiscernables de la méningite bactérienne, de nombreux cliniciens ne peuvent pas faire une distinction claire dans la présentation de la maladie, et un grand nombre de cas ne sont pas diagnostiqués. Bien que la réaction en chaîne par polymérase soit l'approche diagnostique standard actuelle, il faut plusieurs heures ou jours pour obtenir un résultat et ces tests ne

sont pas disponibles aux niveaux de soins primaires et secondaires dans de nombreux pays pauvres en ressources. En outre, le diagnostic est souvent difficile chez les enfants en raison de découvertes non spécifiques du liquide céphalo-rachidien cellulaire et biochimique. Certains enfants atteints peuvent développer des complications neurologiques ou systémiques, entraînant une hospitalisation prolongée, augmentant le risque de décès évitables et les dépenses de santé. Cette revue se concentre sur l'épidémiologie, la présentation et le diagnostic de la méningite à entérovirus et parechovirus, mettant en évidence les défis du diagnostic et les rôles potentiels des tests virologiques sur place dans le LCR dans l'amélioration de la qualité des soins aux patients pédiatriques. Les informations fournies devraient contribuer à la détection précoce des cas, garantissant ainsi d'éviter les antibiotiques inutiles, des complications minimales, une courte période d'hospitalisation et une réduction des coûts associés aux soins de santé.

**Mots clés:** méningite aseptique; Entérovirus; Parechovirus; Défi diagnostique; Test de virologie sur place; Enfants

## Introduction:

Despite advances in antimicrobials, infections of the central nervous system (CNS) remain a major cause of many life-threatening disease conditions, with aseptic meningitis at the forefront, especially in the paediatric age group. Aseptic meningitis (AM) is an acute inflammation of meninges of the brain and spinal cord in which the cerebrospinal fluid (CSF) is negative for bacteria (1). AM is specifically caused by Enteroviruses (EVs) and Parechoviruses (PeVs), however, viruses such as Herpes simplex virus types I & II (HSV I & II), Varicella zoster virus (VZV), Adenovirus (ADV), Rhinovirus (RHV), Epstein Barr virus (EBV), Cytomegalovirus (CMV), Mumps virus, Human herpesvirus 6 (HHV 6) and HIV have been implicated (2-11). Also, there are non-viral causes such as drugs (12-14), parasites, fungi, and inflammatory diseases (7-9). EBV and *Cryptococcus* are recovered mostly in immunocompromised individuals (9).

EVs and PeVs are emerging pathogens that constitute two important genera (Enterovirus and Parechovirus) of the *Picornaviridae* family. The family of picornaviruses consists small ( $\approx 30\text{nm}$ ), non-enveloped viruses containing single-stranded, linear, positive-sense RNA, with several important human and animal pathogens including Polio viruses (15,16). Enterovirus genus consists of 15 species [EV: (A-L) and *Rhinovirus* (RV: A-C)], and over 100 serotypes have been described (17,18). The genus Parechovirus contains four species; Parechovirus A, formally Human Parechovirus (HPeV), Parechovirus B (formerly Ljungan virus infecting rodents), Parechovirus C (Sebokele virus), and Parechovirus D (Ferret Parechovirus). Nineteen different genotypes of PeV-A have so far been described and their count is still on the increase (16,19-21).

Because of their pathogenic potential, increasing frequency of detection (even among healthy individuals), and the ability to cause severe infections, EVs and PeVs attracted more attention and became relevant globally (19-27). But, without clear distinction in clinical

presentation and non-specific CSF findings, diagnosis of AM is rarely considered, and only when the first line of care (usually antibiotic therapy) fails (28-32). The situation is worsening in countries with poor laboratory diagnostic services and inadequate intensive care facilities.

The on-site CSF virology tests eliminate the technicalities of sample preparation and processing and produce results in a matter of minutes. They offer a superior advantage in patients' management through early recognition of cases and exclusion of other suspected pathogens. Reduction in the use of unnecessary antibiotics, expendable costly investigations, as well as guiding investigations and follow-up for potential complications in severely affected children are among added benefits (29,31,33). In this review, we provided a summary of current knowledge on Enterovirus and Parechovirus meningitis, with emphasis on the paediatric age group.

## Methodology:

This article is a narrative review of Enterovirus and Parechovirus meningitis available in the literature. Original and review articles published in English Language were searched for on PubMed, Embase, Scopus, and Google Scholar. Articles reporting information relating to the biology and classification of Enterovirus and Parechovirus, epidemiology, clinical presentations, and laboratory diagnosis of meningitis caused by these viruses, as well as the diagnostic challenges, and the potential roles of on-site CSF virology tests were retrieved and reviewed.

## Epidemiology of Enteroviruses and Parechoviruses

Human EVs and PeV-A are ubiquitous, transmitted mainly by direct contact with respiratory secretions, droplets, nasal discharge, sputum, saliva, or faeces, from symptomatic or asymptomatic carriers, within a family, in schools, hostels, and chronic care facilities (34-39). Nosocomial (40,41), and transplacental

transmission (24,42) have been reported. They cause several diseases in humans (sporadic and endemic) and have the potential for pandemic spread (25,43,44). However, the exact disease incidence is not known. Reports vary by country, demographic characteristics, and virus genotype. EVs typically infects older children and adults while the PeVs predominate in neonates and infants (20,21,31,38,45-52). A pooled-data from Japan, Hong Kong, Denmark, Finland, Netherlands, the USA, and Malawi revealed a prevalence of  $\approx 2\%$  for PeV-A in children with suspected viral infections (35,44,52). In under-developed nations, data are limited because the diagnostic facilities are difficult to operationalize and only restricted to referral laboratories. In tropical Africa, reliable

data are only available in countries with good research-link (9,18,19,30,36,39,52-59).

From a recent study in the UK and the Republic of Ireland, the incidence of EV-PeV meningitis was twice that of bacterial meningitis (24). AM is more prevalent during the hot season, usually May to September (10,24,60-63), but can occur all-year-round (10,49,56,63-67). Cases are seen mostly in children less than five years of age (50,63), more males affected than females, and there may be variation in the distribution (30,55,62,63,65). The infection accounts for many admissions into intensive care units, with associated mortality (11,24,29,49,61,68-70). The summary of the detection rates from various countries is shown in Table 1.

Table 1: Detection rates of Enterovirus (EV) and Parechovirus (PeV) in children with suspected meningitis from various countries

Country	Place of study (province/state/city)	Study period	Targeted age (Mean/Median)	Prevalence (%)		Most affected age (Median)	Reference
				EV	PeV		
Australia	Western Australia	Feb-Jul 1992	< 15 years	80/104 (76.9)		< 5 years	(28)
Brazil	São Joaquim da Barra	Dec 1998-Dec 2003	2-6 years	64/294 (21.8)		1-4 years	(64)
	São Paulo	Feb-May 2004	4.5 years	12/23 (52.2)			(118)
Canada	São Paulo	2008-2009	$\leq 5$ years	49/344 (14.2)			(4)
	Alberta, Manitoba, Y	Jan 1998-Dec 1999	< 1 to 18 years	233/802 (29.1)			(119)
China	Yunnan	2009-2010	3-14 years	85/524 (16.2)		< 14	(76)
	Luoding, Guangdong	May-Jun 2012	6 years	75/121 (62)		3-5 years	(74)
Denmark	Shandong	2014		209/927 (22.5)			(63)
	Zhejiang	2002 to 2015		206/1757 (11.7)			(120)
Egypt	Hebei	Jan-Sept 2015	7 months-16 years	89/268 (33.2)			(10)
		2009-2012	< 5 years		30/32 (94)	39 days	(47)
France	Cairo	Jun 2010-May 2012	1.5 months-12 years		56/100 (56)	< 2 years	(30)
	Clermont-Ferrand	Jan 2008-Dec 2009	2-16 years	17/27 (63)		32 days-2 years	(121)
Germany	Clermont-Ferrand	Jun 2015-Oct 2015	$\leq 28$ days-16 years	222/246 (90.2)		2-16 years	(77)
		Jun 2016-Oct 2016					
Greece	Bonn	May 1998-Oct 2008	8 days-17 years	14/327 (4.3)	2/327 (0.6)	< 3 months	(6)
	Hannover, E	2008	0-18 years	100/157 (63.7)		6.8 $\pm$ 3.8 years	(71)
Iran		2013		96/106 (90.6)		7.1 $\pm$ 4.1 years	
	Athens metropolitan	Jan 1994-Dec 2002	1 month-14 years	47/96 (49)		6-12 years	(93)
Italy	Thessaloniki, E	Mar 2003-Apr 2005	21 days-14 years	14/32 (43.8)		7.73 years	(82)
	Athens	2007	0-15 years	105/177 (59.3)		5.2-7.5 years	(83)
Netherlands	Nemazi, Dastgheib	May 2001-May 2002	2 months-15 years	13/102 (12.7)		8 month-12 year	(122)
	Mashhad city	Mar-Sept. 2007	< 1-month -17 years	21/58 (36.2)		5.7 years	(70)
Palestine	Shiraz	May 2007-Apr 2008	2 months-15 years	13/65 (20)		1-5 years	(3)
	Tehran	2007-2012	< 8 years	275/366 (75.1)	154/366 (42)	20.17m: > 1year	(84)
Russia	Tehran	2009-2011	< 8 years	24/115 (20.87)	51/115 (44)	20.12m: 26.67m	(92)
	Northern Iran	Jun 2014: Mar 2015	6 months-13 years	9/50 (18)		2-5 years	(123)
South Africa	San Matteo, Pavia	Jan 2010-Oct 2013	< 30 days	4/60 (6.7)			(61)
	Tilburg and Breda	Mar 2008-Aug 2011	0-16 years	75/141 (53.2)	16/141 (11.3)	50 days:34 days	(45, 124)
South Korea	West Bank	July 2012- Nov 2015	< 1-10 years	62/324 (19.1)		< 1 year	(88)
	Northern West Bank	Mar-Oct 2017	0-92 months	54/249 (21.7)		0-92 months	(62)
Tunisia	West Siberia	2012-2013	1-14 years	70/143 (50)			(86)
	Mosel Bay, W. Cape	Dec 2015-Jan 2016	< 10 years	43/63 (68.3)		2-4 years	(55)
Turkey	Chungcheong	Jun-Oct 2008	$\leq 16$ years	60/140 (42.9)		2 m-13 years	(125)
	Jinju	Feb 2012-Sept. 2013	1-5 years	20/267 (0.7)	9/267 (3.4)	1.4 m:7.8 m	(75)
UK	Monastir & Mahdia	Jan 2011-Jan 2013	< 28 days-16 years	14/143 (9.8)		2-16 years	(85)
	Ankara	Jun 1999-Dec 2004	< 1-14 years	104/612 (17)		5.6 $\pm$ 3.4year	(2)
USA	London	2008-2012	< 1 years		30/40 (75)	< 90 days	(31)
	Cornwall	2012-2015	$\leq 5$ years	20/98 (20.4)		< 5 months	(60)
UK & Ireland		2016	$\leq 5$ years	27/140 (19.3)	14/140 (10)	< 5 m: < 2 m	
	England	2016	3-150 days		104/106 (98)	< 90 days	(48)
USA	Leicester	Feb 2014-Aug 2017	29-102 days	131/163 (80.4)	32/163 (19.6)	< 90 days	(49)
	Wales & North Ireland	Jul 2014-Aug 2015	< 90 days	668/703 (95)	35/703 (5)	34 days	(24)
	8 Regions	Feb-Sept. 2014	< 2 months-17years	47/639 (2.7)	12/299 (4)	< 2 months	(8)

Y = Ontario, Quebec, Scotia, British Columbia; C = Düsseldorf; Erlangen-Nürnberg; Ludwigsburg; Heidelberg; E = Drama, Patra, Larisa & Bolos

Socio-demographic factors such as age, gender, season, and study design accounted for substantial disparity in disease prevalence, in countries, and regions within the same country, with studies mostly on EVs. Estimates indicated variation in peak age of the infection, but mostly within the first five years of life ( $\leq 5$  years for EVs versus  $< 3$  months for PeVs). It is important to note that symptoms in neonates are subtle and not different from sepsis-like illnesses, which leads to a serious challenge in diagnosis and reporting, suggesting the need for high-quality surveillance to optimize detection, as country-specific estimates are crucial tools to improve diagnostics and therapeutics for these infections.

#### **Prevailing genotypes/serotypes of EVs and PeVs involved in AM and other infections**

On specific serotypes, Coxsackievirus B5 (CVB5), Coxsackievirus A9 (CVA9), Echovirus 6, 9, and 30 and CVB3 are the most commonly reported serotypes from AM and other clinical infections (49,55,63,64,71-78). Other serotypes recovered include Echovirus 1, 3, 4, 5, 7, 11, 13-16, 18, 20, 25, 27, 32 and 33; CVB1, CVB2, CVB4, CVB6; CVA2, CVA4-6, CVA10, CVA15, CVA16, CVA21 and EV71 (6, 10,28,45,49,55,56,60-67,71-73,75,76,79-89). On the other hand, PeV-A3 is the mostly recovered genotype (31,47-50,60,90,91) while other genotypes recovered are PeV-A 2 and 1 (47,92).

#### **Clinical presentations of meningitis caused by EVs and PeVs and complications**

Usually, there is no clear distinction in clinical presentations of meningitis caused by EV, PeV, or bacteria agents. Fever is the most common symptom and is usually moderate to high grade. Nausea, vomiting, diarrhea, poor feeding, irritability, convulsions, headache, and altered level of consciousness are notable symptoms (24,31,56,60-64,71,77,82,93-95). Headache, vomiting, neck rigidity, and lethargy are pronounced in children with EV meningitis (77, 94,96). Patients may present with typical features of upper respiratory tract infections, and maculopapular rashes mostly in those with PeV infections (31,60,97).

The severity and risk of developing complications depend on the virus type, patient age, and the interval between disease onset and presentation to the physician (31, 32,94,98,99). Abnormalities of the white matter and neurodevelopmental delays are the common sequelae (24,31,32,47,50,51,91,98,

100,101). Subdural hemorrhage, coronal infarction, cystic encephalomalacia, periventricular leukomalacia, and ventricular dilatations are specific complications. Such patients may manifest with failure to thrive (FTT), recurrent seizures, visual impairment, and global hypotonia (11,24,31,49,91,102). Hyponatremia due to the syndrome of inappropriate antidiuretic hormone secretion (SIADS) has been reported (70). Shaker and Abdelhamid reported 50% mortality in patients who tested positive for EV (30), while some other studies also reported considerable mortalities (24,31,70).

#### **Laboratory diagnosis of EVs/PeVs infections and challenges**

Laboratory testing is essential for the definitive diagnosis of infectious meningitis, particularly in the young infant, because clinical disease presentation lacks predictive value. CSF pleocytosis and elevated protein are the recommended criteria for diagnosis, however, many studies showed varied results (10, 24,28-32,56,60,73,75,88,91). With contradictory reports of other markers such as C-reactive protein (CRP), lactate, mononuclear, or polymorphonuclear cells counts (24,91,103, 104), there could be uncertainty in diagnosis and treatment. Therefore, it should be noted that with the positive signs and symptoms of meningitis, the absence of CSF pleocytosis, elevated protein, or other markers of interest does not rule out EV or PeV meningitis.

The mainstay of the diagnosis is molecular-based assays on appropriately collected-samples. Cell culture is one of the most popular methods for virus isolation, evidenced by the cytopathic effect (CPE), which alter specific characteristics of the cells. However, cultures are intensive and some viruses (most especially PeVs) do not replicate in commonly used cell lines. Thus, researchers nowadays are reluctant to use cultures and tend to adopt PCR techniques for relative simplicity and short window for results (21,23). Recently, we evaluated the diagnostic sensitivity of cell culture, real time RT PCR, and nested RT PCR for EVs and PeVs, and real time RT PCR demonstrated the highest sensitivity and negative predictive value (NPV), particularly for EVs (unpublished work). Specifically, real-time PCR is the recommended test for EVs and PeVs in all clinical specimens (21,23). The PCR assay relies on the extraction and purification of nucleic acid, then exponential amplification of the target sequence, using a thermostable polymerase and specific primers.

Unfortunately, most tropical and sub-

tropical countries cannot establish qualitative molecular-based diagnostic tests in basic healthcare settings. This contributes to the enormous burden of ill health as infectious diseases represent the major cause of death in most of the countries. Another fundamental problem is the lack of incorporation of EVs and PeVs among the list of priority infections. This significantly deterred identification of the pathogens even in developed nations. Therefore, misdiagnosis and failure to treat a serious infection or wasting expensive treatment on those not infected remain a serious obstacle. Even without targeted treatment, early identification of infections has economic benefits, which include stopping the use of unnecessary antimicrobial drugs, minimizing expendable investigations, and shortening the length of hospital admissions (24,105,106).

#### **On-site CSF virology tests and management of EV and PeV infections**

The WHO has established the ASSURED (Affordable, Sensitive, Specific, User friendly, Rapid, and robust, Equipment-free, and Deliverable to end-users) criteria (107) for diagnostics in a resource-poor setting. This aimed at providing better management of diseases through immediate delivery of results and a rapid record of the disease status, improve clinical decision-making. The cost-effective on-site CSF virology tests employ reverse-transcriptase polymerase chain reaction to rapidly identify the presence of viral RNA in CSF of a suspected infected individual. These offer superior advantages over conventional nested PCR and real-time PCR by providing prompt identification of the pathogen, guide selection of therapy, and minimize complications. Added advantages include short time window for results (rapid turnaround time), antimicrobial stewardship through appropriate prescribing practice, reduction in financial costs, and improve patient outcomes (8,9,108-114). Additionally, the WHO recommended criteria of the physical structure and human resources as key elements of a virology laboratory (115) can be adjusted to suit the desired need in a particular setting, hence, guaranteeing the feasibility of carrying out these tests in most of our local clinics and hospitals.

Currently, there are no antiviral agents licensed for the treatment of EVs and PeVs infections, but vaccines are available only against PVs, and China licensed an EV71 vaccine in December 2015 (116). Researchers are currently exploring the potential benefits of intravenous immunoglobulins (IVIGs), predni-

solone, and other compounds in the management of EVs and PeVs infections. Of recent, a compound 2'-C-methylcytidine, an inhibitor of viral polymerase, demonstrated promising results against PeA-1 and 3 *in-vitro* (117).

#### **Conclusion:**

Enteroviruses and Parechoviruses are the leading causes of aseptic meningitis, and there exist indistinguishable presentations with bacterial meningitis, while CSF pleocytosis and biochemical tests show limited roles in diagnosis. The disease prevalence varies in different geographical regions, mainly affected by socio-demographic factors, and E9, 30, 6, 16, CVB5, CVA9, CVB3, and PeV-A3 are commonly isolated virus serotypes. Although the WHO recommends syndromic management in settings with limited access to laboratory diagnostic services, the economic burden of treating common causes of the syndrome is outrageous and merely impossible in local settings. With the evolution of high precision point-of-care CSF virology tests, stockholders should explore their benefits to optimize the quality of care in children with EVs and PeVs meningitis, most especially in resource-poor settings.

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#### **Conflict of interest:**

Authors declare no conflict of interest.

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**Original Article****Open Access****Demographic attributes of COVID-19 patients in an Infectious Disease Center of Nigeria**

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**Abstract:**

**Background:** As part of our contribution to the growing pool of knowledge on the prevention and control of the COVID-19 pandemic, this study describes the demographic features of patients with COVID-19 hospitalized at Infectious Disease Center (IDC), Olodo, Ibadan, Oyo State, Nigeria.

**Methodology:** This was a descriptive cross-sectional study of COVID-19 patients whose data were collected during admission between April 27, 2020 and June 20, 2020. SARS-CoV-2 infection was diagnosed on nasopharyngeal specimen using a real-time reverse transcription-polymerase chain reaction (rRT-PCR) assay. Data were analysed using the Statistical Package for Social Sciences (SPSS Inc., USA) version 20.0

**Results:** Among 131 patients, 58% were between age 18 and 35 years, 48.1% were employees of private establishments, and 64.1% were males. High proportion (84.3%) of the patients spent less than 14 days on admission. As at June 20, 2020, the overall COVID-19 mortality in the IDC was 0.0%.

**Conclusion:** This study concluded that COVID-19 was common among male Nigerians, those working in private establishments, and those aged 18-35 years. Future researches on COVID-19 in Nigeria must put gender and age into consideration.

**Keywords:** SARS-COV2; COVID-19; age; gender; occupation

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**Attributs démographiques des patients atteints de COVID-19 dans un centre de maladies infectieuses du Nigéria**

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## Abstrait:

**Contexte:** Dans le cadre de notre contribution au pool croissant de connaissances sur la prévention et le contrôle de la pandémie COVID-19, cette étude décrit les caractéristiques démographiques des patients atteints de COVID-19 hospitalisés au Centre des maladies infectieuses (IDC), Olodo, Ibadan, État d'Oyo, Nigéria.

**Méthodologie:** Il s'agissait d'une étude transversale descriptive de patients atteints de COVID-19 dont les données ont été collectées lors de l'admission entre le 27 avril 2020 et le 20 juin 2020. L'infection par le SRAS-CoV-2 a été diagnostiquée sur un échantillon nasopharyngé à l'aide d'une transcription inverse en temps réel-Test de réaction en chaîne par polymérase (rRT-PCR). Les données ont été analysées à l'aide du Statistical Package for Social Sciences (SPSS Inc., USA) version 20.0.

**Résultats:** Parmi 131 patients, 58% avaient entre 18 et 35 ans, 48,1% étaient des employés d'établissements privés et 64,1% étaient des hommes. Une forte proportion (84,3%) des patients ont passé moins de 14 jours à l'admission. Au 20 juin 2020, la mortalité globale par COVID-19 dans l'IDC était de 0,0%.

**Conclusion:** Cette étude a conclu que le COVID-19 était courant chez les hommes Nigériens, ceux travaillant dans des établissements privés et ceux âgés de 18 à 35 ans. Les futures recherches sur le COVID-19 au Nigéria doivent prendre en compte le sexe et l'âge.

**Mots clés:** SRAS-COV2; COVID-19; âge; le sexe; occupation

## Introduction:

Coronavirus disease-2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-COV2) is a viral disease that is of great global public health concern. Coronavirus (COV) is one of the major viral pathogens that primarily targets the human respiratory system. Person-to-person transmission of coronaviruses occurs primarily via direct contact or through droplet spread when an infected individual coughs or sneezes (1). The binding of the receptor-binding domain (RBD) of the virus spike proteins to the host angiotensin converting enzyme-2 (ACE2) cellular receptor, fuses SARS-COV-2 with the host cellular membrane (2,3) to initiate and establish the disease. Therefore, measures to reduce person-to-person transmission of SARS-COV-2 in COVID-19 needs to be implemented to control the current pandemic through primary protection particularly of the susceptible populations such as the elderly, children, health care providers, and people with underlying medical conditions (1).

The symptoms of COVID-19 appear after an incubation period of approximately 5.2 days while the period from the onset of COVID-19 symptoms to death ranged from 6 to 41 days with a median of 14 days, but this is highly dependent on the age of the patient and status of the patient's immune system (4). This period has been reported to be shorter among patients above 70 years of age compared to those under the age of 70 years (4). These findings underscore the need to

have a good understanding of the demographic profiles of COVID-19 patients in different populations.

The chronology of coronavirus infections in Nigeria as revealed by the Federal Ministry of Health was that the first confirmed case of COVID-19 in Lagos State, Nigeria was registered on the 27th of February 2020, although the outbreak in China began in January 2020 (5). On 21st March 2020, the first case of COVID-19 was confirmed in Ibadan, Oyo State, Nigeria which brought the tally of confirmed COVID-19 cases in Nigeria at that time to 23 (6). By the 30th of July 2020, Oyo State had a total 2,713 laboratory confirmed cases, 1,451 cases on admission at various isolation homes or health outposts, 1,259 cases had been discharged, and 27 deaths recorded (case fatality rate of 0.99%). In Nigeria as a whole on the same date, there were 42,689 confirmed cases, 22,331 active, 19,270 discharged, and 878 deaths (case fatality rate of 2.05%) (7).

According to a previous study, social and economic factors were reported to have potential impact on infectious disease dynamics (8). Fang et al., (9) reported that medical staff density had significant positive influence on occurrence of SARS infection in mainland China. School education and living conditions were associated with hospitalization during the 2009 H1N1 influenza pandemic (10). Through respiratory droplets as the major means of transmission of SARS-COV-2, COVID-19 now spreads easily and sustainably in the Nigerian community (11,

12), and previous studies have opined that socio-economic factors such as occupation might have impacts on the COVID-19 spread.

Symptomatic COVID-19 patients are usually managed in isolation centers where varied management modalities including supportive and definitive therapies are administered. One of such centers in Oyo State is the Infectious Disease Center located in Olodo, Ibadan, Nigeria. This study describes the demographic characteristics of COVID-19 patients treated at the center over a period of approximately two months.

## Materials and method:

### Study setting

The study was conducted in Ibadan, the capital city of Oyo State, Nigeria, located about 100 miles (160 km) from the Atlantic coast. It is one of the most populous cities in the country and the economic activities of Ibadan include agriculture, commerce, handicrafts, manufacturing, and service industries. Ibadan is an important commercial centre having many markets.

### Study design, subjects and data collection

This was a descriptive cross-sectional study of a total of 131 patients with COVID-19 treated at the Infection Disease Center (IDC), Olodo, Ibadan, who were enrolled into the study between April 27, 2020 and June 20, 2020. The COVID-19 cases with clinical symptoms of dry cough, high fever, sore throat and/or shortness of breath, were confirmed by detection of SARS-COV2 nucleic acid using real-time reverse-transcriptase polymerase chain reaction (rt-PCR) assay on

nasal and pharyngeal swab specimens following recommended guidelines (13).

Data were collected using pre-tested questionnaire which contains information on age, gender, pre-existing conditions, occupation and level of education. The date of admission was subtracted from the day of discharge to obtain the number of days on admission. Before the commencement of the study, the approval (UI/EC/20/0233) was obtained from the University of Ibadan/ University College Hospital (UI/UCH) Joint Ethics Review Committee. In addition, informed consent was obtained from the study participants.

### Statistical analysis

Data obtained were analysed using Statistical Package for Social Sciences (SPSS Inc., USA) version 20.0 and the descriptive analyses of data were presented as frequency and percentages.

## Results:

The demographic characteristics of the 131 COVID-19 participants are shown in Table 1. High frequencies of the participants were between the ages of 18 and 35 years (58%), were largely employees of private establishments (48.1%), and mostly males (64.1%). One patient each had hypertension, diabetes mellitus, sickle cell anaemia and stroke, as underlying medical conditions. A high percentage (84.3%) of the patients spent less than 14 days on admission, and mortality was 0.0% during the period of study. The number of patients admitted and discharged increased weekly till week 4 and 5 respectively in the center (Fig 1).

Table 1: Demographic characteristics of COVID-19 patients in Infectious Disease Center (IDC), Olodo, Ibadan, Nigeria

Characteristic variables	Frequency (n=131)	Percentage
<b>Age group (years)</b>		
<18 years	4	3.1
18-35 years	76	58.0
36-55 years	36	27.5
>55 years	15	11.5
<b>Gender</b>		
Male	84	64.1
Female	47	35.9
<b>Occupation</b>		
Unemployed	20	15.3
Self employed	28	21.4
Private Companies	63	48.1
Civil servant	20	15.3
<b>Days on admission of 96 discharged patients</b>		
≤7 days	8	8.3
8-14 days	73	76.0
>14days	15	15.6
<b>Co-morbidities (n=4)</b>		
Stroke	1	0.7
Hypertension	1	0.7
Sickle cell anaemia (HbSS)	1	0.7
Diabetes mellitus	1	0.7
<b>Mortality rate at IDC, Olodo, Ibadan</b> (April 27- June 20, 2020)	0	0.0



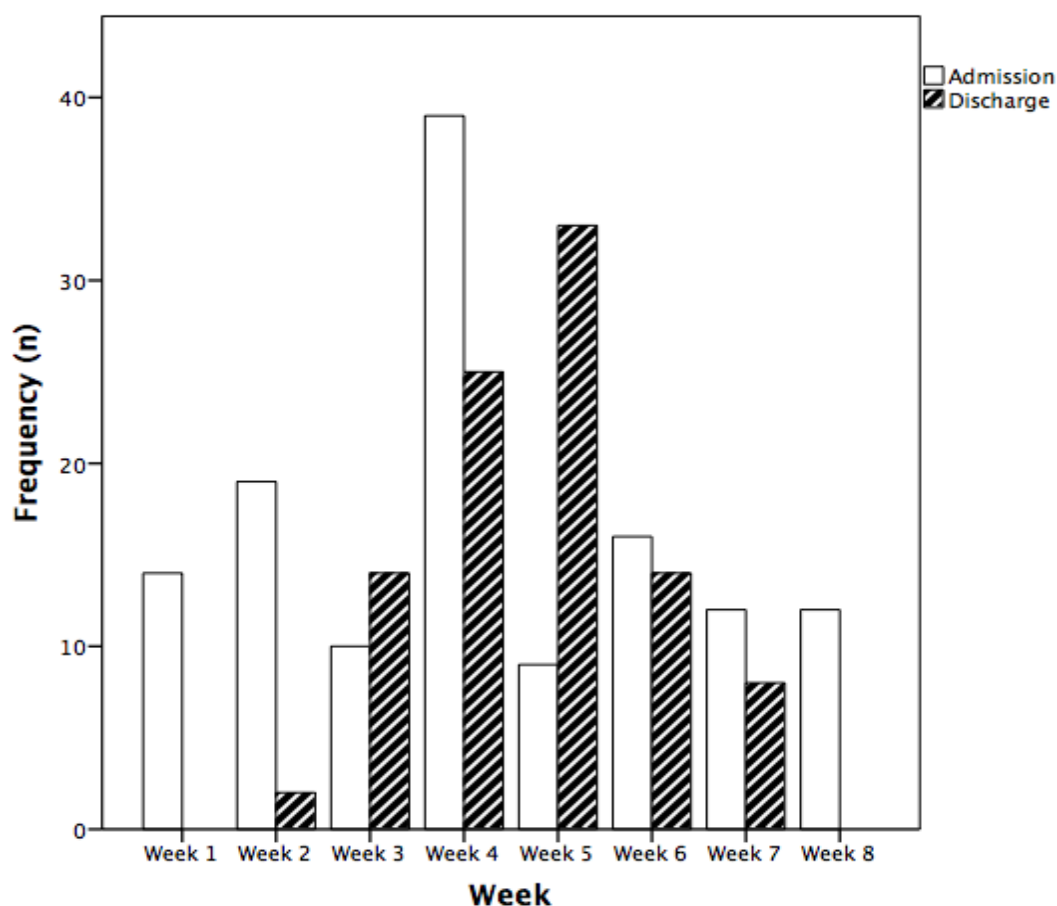


Fig 1: Weekly distribution of admission and discharge rates in Infectious Diseases Center, Olodo, Ibadan, Nigeria

## Discussion:

SARS-CoV-2 belongs to RNA family of coronavirus causing severe respiratory diseases in humans (14). Epidemiological studies show that the SARS-CoV-2 is more contagious than SARS-CoV but its lethality is said to be less than that of SARS or MERS (1,15-18). However, the mortality of COVID-19 outbreak is still relatively high and the pandemic is ongoing in several countries of the world. The World Health Organization (WHO) on March 10, 2020 reported mortality rates for COVID-19 of 3.88% in China, 5.05% in Italy, 4.03% in the United States, 0.75% in South Korea, 3.81% in Iran, 2.73% in Spain, 2.14% in France, 1.75% in Japan, 0.93% in Britain, and 0.18% in Germany (1,15-18). As at July 30, 2020, Oyo State in southwest Nigeria had reported 27 COVID-19 related deaths out of 2,713 laboratory confirmed cases (case fatality rate of 0.99%), and 1,259 discharges (19). On the same date, Nigeria recorded 878 COVID-19 related deaths out of a total of 42,689 confirmed cases (case fatality rate of 2.05%), with 19,270 discharges.

The predominant age-group affected, according to the Nigeria Center for Disease

Control (NCDC) data was 31 to 40 years, and in relation to gender distribution, males were more frequently infected than females. With the increasing number of confirmed cases in the country, the NCDC believed that in the absence of a vaccine, compliance with infection prevention and control (IPC) measures remains the most effective intervention to control the COVID-19 pandemic (20). Also, many experts believed that SARS-CoV-2 may coexist with humans for a long time (15), therefore, special attention should be paid to potential COVID-19 infection risks (21). Tracking the epidemiological attributes of COVID-19 patients in each locality will hence be highly required.

In this study, a higher proportion of patients with COVID-19 were males within the active age of 18 and 35 years. This observation supports the report that the most affected age groups in Nigeria are those in 31 to 40 years group (22). Sun et al., (23) also reported similar observation in a part of China. This observation could be due to high outdoor activities in males within this age group which could increase their risks of contracting the virus. Most people in this age group are either in schools, unemployed or engaged in

available petty employment such as selling, driving commercial bicycle, tricycle or taxis. Thus, this group of people are prone to contacting SARS-COV-2 especially when they do not adhere to infection prevention and control measures.

Data from some countries showed similar numbers of COVID-19 cases in women and men, but an increased case fatality in men (24,25). In our study however, no death was recorded for either male or female COVID-19 patients during the study period, but the infection rate was 64.1% in the males compared to 35.9% in the females. Genetics and sex hormone differences determine susceptibility and response to viral infections between males and females, which may be responsible for the gender differences in the incidence and severity of COVID-19 in our study and those of others (26). It has also been reported that exposure to influenza A viruses is often higher in males than females but fatality following exposure is reportedly higher in females (27). The adaptive immune responses to viral infections differ between males and females (28). In the studies by Boissier et al., (29), Xia et al., (30) and Melgert et al., (31), the number and activity of innate immune cells were reportedly higher in females than in males.

In this study, the frequency of COVID-19 was highest among patients working in private companies (48.1%), followed by the unemployed (21.4%). Most of the patients working in private companies are staffs who have to adhere strictly to the rule and regulations of the employers or face the threat of sack. In private companies, gain is maximised and staff are paid based on work done. The high frequency of the disease among the unemployed group may be due to the fact that they are freely mobile in search of means of livelihood. Moreover, this group have limited resources and may have no fund to purchase face masks and hand-sanitizers needed to comply with COVID-19 preventive measures. Some of the them may also have some degrees of immuno-compromise from poor or malnutrition.

Because economic activities are closely related to human behaviour (32,33), they have impacts on the COVID-19 morbidity and mortality (8), which as a result of competition for scarce resources, could facilitate SARS-COV2 transmission via respiratory droplets and contacts. The transmission of COVID-19 via droplets and fomites is greatly enhanced by close contact between infectors and infectees (34). Ecological, biological, and social factors greatly influence infectious diseases dynamics (35). Some social and economic factors that change human behaviour also present challenges for prevention and

control of infectious diseases (36), including COVID-19. The development of the tertiary industry and retail sales needs large number of proprietors and consumers. Areas with total retail sales of consumer goods per unit of the land area indicate that people prefer to purchase in the market in these areas. These influencing factors lead to the risk of the transmission of COVID-19 in the commercial activity as seen in our study which showed that private workers were more frequently infected.

The number of admissions increased from the beginning of our recruitment. This was due to a number of factors such as; increased community transmission, increased numbers of screening centers, initial stigma related to COVID-19 had largely been ignored by the populace which aided voluntary reporting to screening centers, massive education, and enlightenment of the populace with a clear message that COVID-19 was not a "death-sentence". There was no case of COVID-19 re-infection in the center during the period of the study, while the number of discharged cases increased weekly. This observation may have been related to effective management, and non-fatal nature of SARS-COV-2 strain, which may be responsible for the mild nature of the COVID-19 disease without complications, seen in most of the patients at the time of admission. These may also account for zero mortality rate of COVID-19 cases in the center during this period.

The COVID-19 symptoms have been reported to resolve after about 10 days (37). However, viral shedding may continue in spite of symptoms disappearance (37,38), and COVID-19 RNA viral shedding can persist for about 18 days in the nasopharynx or 19 days in the faeces (39). Mild and asymptomatic cases tend to shed the virus for 10 days (8–15 days) after symptom resolution (40), with 90% resolving after 10 days and nearly all cases resolving after 15 days (38). These observations were consistent with findings in our center where most of our patients stayed between 8 and 14 days on admission. However, multi-centered studies will be required to have a better understanding of the dynamics of the COVID-19 pandemic with regards to demography, disease burdens (mortality and morbidity) and risk factors associated with different stages of COVID-19.

## Conclusion:

In conclusion, our study showed that COVID-19 was more frequent among male Nigerians, those working in private establishment and those in active age group (18-35 years), thus providing ideal about the likely

SARS-CoV-2 reservoir populations to be targeted for control measures in the ongoing COVID-19 pandemic in Nigeria. Our findings also emphasised the impact of age, occupation and gender on the incidence or case fatality of COVID-19, thus tailoring treatment accordingly or giving the basis for further studies, which should put gender and age analyses into consideration.

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## Conflicts of interest:

No conflict of interest is declared.

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## Original Article

## Open Access

***Klebsiella pneumoniae* producing extended spectrum  $\beta$ -lactamase in Regional Military University Hospital of Oran, Algeria: antibiotic resistance, biofilm formation, and detection of *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> genes**\*<sup>1</sup>Benbrahim, C., <sup>1</sup>Barka, M. S., <sup>2</sup>Benmahdi, L., <sup>3</sup>Zatout, A., and <sup>1</sup>Khadir, A.<sup>1</sup>Laboratory of Applied Microbiology in Food, Biomedical and Environment (LAMAABE), Department of Biology, Faculty of Nature and Life, Earth and Universal Sciences, Abou Bekr Belkaid University, 13000 Tlemcen, Algeria<sup>2</sup>Laboratory of Microbiology, Regional Military University Hospital, Oran, Algeria<sup>3</sup>Laboratory of Microbiology and Plant Biology, Department of Biological Sciences, Faculty of Natural Sciences and Life, University of Abdlhamid Ibn Badis, Mostaganem, Algeria\*Correspondence to: [chahla.benbrahim@univ-tlemcen.dz](mailto:chahla.benbrahim@univ-tlemcen.dz)**Abstract:****Background:** *Klebsiella pneumoniae* is a bacterial pathogen commonly associated with severe nosocomial and community acquired infections especially through the acquisition of extended spectrum  $\beta$ -lactamases (ESBL) and biofilm formation capacity. The objectives of this study are to determine the prevalence of *K. pneumoniae* ESBL (KP-ESBL)-producing isolates in the Regional Military University Hospital of Oran (HMRUO) Algeria, characterize their antibiotic resistance profile, genetically detect *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes, and evaluate their biofilm formation capacity.**Methodology:** Different clinical specimens including blood, cerebrospinal fluids, urine and catheter, pus, perirectal abscess, and surgical wounds were collected from patients with suspected clinical infections in different units and departments of the hospital. The specimens were cultured on Blood, MacConkey and CLED agar (for urine only) plates and incubated aerobically for 24 hours at 37°C for preliminary identification of bacteria using conventional colony morphology, Gram stain reaction, and disk diffusion test for antibiotic susceptibility testing (AST). Confirmation of isolates, antibiogram, minimum inhibitory concentration (MIC) and detection of resistance phenotypes, were carried out by the automated Vitek 2 (BioMérieux) identification and susceptibility method. ESBL production was confirmed by the synergy and combination disk tests. ESBL genes were detected by conventional simplex PCR and biofilm formation was detected by the tissue culture plate (TCP) method.**Results:** A total of 630 patients' clinical samples (one sample per patient) were processed. *Klebsiella pneumoniae* was isolated in 40 (6.3%) samples, and 15 of these (37.5%) produced ESBL. In the disk diffusion AST assay, all 40 *K. pneumoniae* isolates were resistant to ampicillin and ticarcillin while all 40 isolates were sensitive to cefoxitin, imipenem and ertapenem. KP-ESBL producing isolates were more frequently recovered from intensive care unit (33.3%) and from urine (46.7%) samples. Group 1 *bla*<sub>CTX-M</sub> genes were detected in 13 of the 15 (86.7%) KP-ESBL isolates, and 46.7% of these isolates were moderate biofilm producers.**Conclusion:** There is need for health departments to put in place preventative measures through regular surveillance of these resistant pathogens and initiating appropriate infection prevention and control strategies to limit their spread in Algerian hospitals and worldwide.**Keywords:** *Klebsiella pneumoniae*, ESBL, biofilm, PCR, antibacterial resistance

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***Klebsiella pneumoniae* productrice de lactamase spectre tendu dans l'hôpital universitaire militaire régional d'Oran, Algérie: résistance aux antibiotiques, formation de biofilm et détection des gènes *bla*<sub>CTX-M</sub> et *bla*<sub>TEM</sub>**\*<sup>1</sup>Benbrahim, C., <sup>1</sup>Barka, M. S., <sup>2</sup>Benmahdi, L., <sup>3</sup>Zatout, A., et <sup>1</sup>Khadir, A.<sup>1</sup>Laboratoire de Microbiologie Appliquée à l'Agroalimentaire au Biomédical et à l'Environnement, Département de Biologie, Faculté des sciences de la nature et de la vie de la terre et de l'univers, Université Abou Bekr Belkaid

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## Abstrait:

**Contexte:** *Klebsiella pneumoniae* est un pathogène bactérien communément associé aux infections nosocomiales et communautaires sévères, en particulier par l'acquisition de  $\beta$ -lactamases à spectre étendu (ESBL) et la capacité de formation de biofilm. Les objectifs de cette étude sont de déterminer la prévalence des isolats de *K. pneumoniae* producteurs de  $\beta$ LSE (KP- $\beta$ LSE) au CHU d'Oran (HMRUO) Algérie, caractériser leur profil de résistance aux antibiotiques, détecter génétiquement les gènes *bla*<sub>TEM</sub> et *bla*<sub>CTX-M</sub>, et évaluer leur capacité de formation de biofilm.

**Méthodologie:** Différents échantillons cliniques, y compris du sang, des liquides céphalo-rachidiens, de l'urine mictionnelle et du cathéter, du pus, des abcès périrectal et des plaies chirurgicales ont été prélevés des patients suspectés d'infections cliniques dans différentes unités et départements de l'hôpital. Les échantillons ont été cultivés sur des milieux de culture: deglose au sang, MacConkey et CLED (pour l'urine uniquement) et incubés en aérobie pendant 24 heures à 37°C pour l'identification préliminaire des bactéries en utilisant la morphologie conventionnelle des colonies, la coloration de Gram et le test de diffusion sur disque pour les tests de sensibilité aux antibiotiques (AST). La confirmation des isolats, l'antibiogramme, la concentration minimale inhibitrice (CMI) et la détection des phénotypes de résistance ont été réalisés par la méthode automatisée d'identification et de sensibilité sur Vitek 2 (BioMérieux). La production de  $\beta$ LSE a été confirmée par les tests de synergie et de double disques. Les gènes de  $\beta$ LSE ont été détectés par PCR simplex conventionnelle et la formation de biofilm a été détectée par la méthode de la plaque de culture tissulaire (TCP).

**Résultats:** Un total de 630 échantillons cliniques de patients (un échantillon par patient) ont été traités. *Klebsiella pneumoniae* a été isolé dans 40 échantillons (6,3%) et 15 d'entre eux (37,5%) ont produit des  $\beta$ LSE. Dans le test AST à diffusion sur disque, tous les 40 isolats de *K. pneumoniae* étaient résistants à l'ampicilline et à la ticarcilline, tandis que les 40 isolats étaient sensibles à la céfoxitine, à l'imipénème et à l'ertapénème. Les isolats producteurs de KP- $\beta$ LSE ont été plus fréquemment récupérés dans les unités de soins intensifs (33,3%) et dans les échantillons d'urine (46,7%). Les gènes *bla*<sub>CTX-M</sub> du groupe 1 ont été détectés dans 13 des 15 isolats de KP- $\beta$ LSE (86,7%), et 46,7% de ces isolats étaient des producteurs de biofilm modérés.

**Conclusion:** Il est nécessaire que les services de santé mettent en place des mesures préventives grâce à une surveillance régulière de ces pathogènes résistants et à la mise en place de stratégies appropriées de prévention et de contrôle des infections pour limiter leur propagation dans les hôpitaux algériens et dans le monde.

**Mots clés:** *Klebsiella pneumoniae*,  $\beta$ LSE, biofilm, PCR, résistance antibactérienne

## Introduction:

Bacterial antibiotic resistance (ATB) is constantly evolving. For over 30 years, antibiotic resistance among enterobacteria to the third-generation cephalosporins (3GC) has been steadily increasing, notably through the production of extended-spectrum beta lactamases (ESBLs). These enzymes including TEM, SHV, CTX-M and their derivatives confer resistance on enterobacteria to all  $\beta$ -lactam agents with exception of cephamycins and carbapenems (1,2). While ESBL-producing enterobacteria were mostly observed in hospitals with varying isolation frequencies from hospital to hospital, and even from department to department within the same hospital, the diffusion of these multiresistant pathogens into community is of increasing concern. The transmission of genes encoding ESBL, mainly through plasmids, is responsible of their rapid dissemination and thus account for the increased prevalence of ESBL-producing bacteria worldwide, which constitute a major public health problem (1,3,).

*Klebsiella pneumoniae* is an important opportunistic pathogen causing nosocomial and community associated infections (4). The

organism is part of the normal microflora of the intestine and commonly responsible for severe infections of the respiratory tracts (hospital acquired and ventilator associated pneumonia), catheter-related urinary tract infection, meningitis, blood stream infections (bacteremia and septicemia), infections of surgical and non-surgical wounds, diarrhea, diseases, prosthetic valve endocarditis, peritonitis, and osteomyelitis (5-11).

The first ESBL-producing *K. pneumoniae* strains were first reported in Europe in 1982 where a new resistance to ceftazidime and aztreonam from plasmid-transmitted  $\beta$ -lactamase enzyme was quickly and easily disseminated to other Gram-negative bacteria including *Escherichia coli*. Since the discovery of these enzymes, they have not stopped growing, and today there are over than 200 different ESBL enzymes. The impact of the ESBL strains is very significant, especially in the intensive care units with a high propensity for epidemic outbreaks. It has been shown in previous European studies that *K. pneumoniae* and *E. coli* were the two most common bacterial species frequently involved in ESBL production (12).

There is need for active surveillance



for ESBL-producing pathogens in high-risk populations using appropriate antimicrobial techniques because these pathogens are generally multiresistant (12,13). ESBL genes are typically carried by large transferable plasmids (85–275kb) on which they are often other associated genes coding for resistance to aminoglycosides, chloramphenicol, sulfonamides, trimethoprim, tetracyclines and fluoroquinolones (12). Although, the  $\beta$ -lactamase inhibitors are used to treat serious infection caused by ESBL-producing *K. pneumoniae*, this should be done with caution because *in vitro* sensitivity does not necessarily predict *in vivo* efficacy (13). Surveys conducted in different countries during specific time periods revealed that once a CTX-M  $\beta$ -lactamase enters a specific geographic area, it becomes predominant, and displace or superimpose itself over TEM and SHV ESBL variants (14).

The main virulence factors contributing to the pathogenesis of *K. pneumoniae* are the capsular polysaccharides and pili type 1 and 3 (6,7). Biofilms formation is another virulence factor. Biofilms are bacterial populations linked by exopolysaccharide matrices at the surface. This matrix of extracellular polymeric substances (EPS) consists mainly of polysaccharides, proteins, lipids and nucleic acids in varying amounts (11). The first strain of *K. pneumoniae* producing biofilms was described in the late 1988 (7). Biofilm-forming bacteria are often observed on the urinary catheter and on the surface of tissues and biomaterials usually at sites of persistent infection, where biofilm formation is a major cause of implant failure, and often limits the lifetime of many permanent medical devices (7,11).

The ability of *K. pneumoniae* to form biofilm provides protection against the host immune system as well as to antibiotics, therefore, biofilms are a major cause of resistance to antimicrobial agents, with resultant high costs of treatment of infections caused by these strains (15). For these reasons, information on ESBL and biofilm producing organisms are required for implementation of preventive procedures and application of infection control measures. The objectives of this study are; to determine the prevalence of ESBL-producing *K. pneumoniae* isolates and their antibiotic resistance profile, genetically detect *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes, and evaluate their capacity to form biofilms.

## Materials and method:

### Study setting and design

This descriptive cross-sectional study was conducted over a period of 5 months (from October 29, 2017 to March 29, 2018) on 630 patients from different units and departments of the Regional Military University

Hospital of Oran, Algeria, whose clinical samples were collected and processed at the Microbiology Laboratory of the hospital.

### Sample and data collection, culture, bacteria isolation and identification

Clinical specimens comprising voided urine (n=333), pus (n=173), blood for culture (n=98), cerebrospinal fluids (n=12), catheter urine (n=8), perirectal abscess (n=4), and surgical wound (n=2) were collected by standard procedures from patients in different departments/units (paediatrics, infectious disease, general surgery, oncology, pneumology, haematology, intensive care unit, urology, re-education, nephrology, child surgery, and psychiatry) of the hospital, and from some external patients. From each patient, socio-demographic information including age and gender, specimen types, service units, and sampling date, were collected into a designed collection form.

Samples were cultured on Blood and MacConkey agar plates (Fluka), with the exception of urine samples which were cultured on Cysteine Lactose Electrolytes Deficient (CLED) agar medium. All culture plates were incubated aerobically for 24 hours at 37°C, and bacteria were preliminarily identified by conventional microbiological methods of colony morphology and Gram reactions, and then purified by successive subcultures on MacConkey agar purity plates. Confirmation of *K. pneumoniae* isolates, antibiogram, minimum inhibitory concentrations (MICs) determination and detection of resistance phenotypes were performed with automated VITEK 2 (BioMérieux) ID and susceptibility platform, using GN and AST-N 233 tapes. The susceptibility results were interpreted according to the recommendations of CLSI (16).

### Synergy test for ESBL production

Synergy test was performed under the same conditions of the antibiogram after detection of resistance phenotypes by VITEK 2 (BioMérieux) ID. Amoxicillin/clavulanic acid (AMC) disk (20/10 $\mu$ g) was placed at 30 mm center to center of ceftazidime (CAZ) disk (30 $\mu$ g), cefotaxime (CTX) disk (30 $\mu$ g), ceftriaxone (CRO) disk (30 $\mu$ g) and aztreonam (ATM) disk (30  $\mu$ g) on MH agar plate that has been inoculated with 0.5 McFarland standards suspension of *K. pneumoniae* isolates. *Klebsiella pneumoniae* ATCC 700603 was used as control. ESBL production results in the appearance of an image of synergy or champagne plug (17).

### Combination disk test for ESBL production

The combination disk test was performed by placing clavulanic acid disk (10 $\mu$ g) and a third generation cephalosporin (3GC) disk at a distance of 30 mm on a MH agar

plate that has been inoculated with 0.5 McFarland standards suspension of *K. pneumoniae* isolates (test), and *K. pneumoniae* ATCC 700603 as control. ESBL production was phenotypically confirmed when the inhibition zone diameter of the 3GC disk tower applied after diffusion of the clavulanic acid disk is  $\geq 5$  mm in relation to the inhibition zone diameter of the 3GC disk tower (17).

#### Molecular detection of ESBL genes

Conventional simplex PCR for the detection of *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> genes was performed in the laboratory of Department of Public Health in Naples, Italy, with specific primers (18-20) as shown in Table 1. The bacterial DNA was extracted by the boiling method (21). PCR was performed in a thermal cycler (Applied Biosystems 2720, California, USA) and the amplification conditions were; initial denaturation at 94°C for 5 mins followed by 30 cycles at 94°C for 25 seconds, annealing at 52°C for 40 seconds, elongation for 72°C for 50 seconds and final elongation at 72°C for 6 minutes. The PCR products were electrophoresed on 1.5% agarose gel, visualized in the UV transilluminator after staining with ethidium bromide, and then photographed. The molecular weight marker (100 bp) was used to determine the sizes of the expected bands (Table 1).

#### Biofilm formation by Tissue Culture Plate method (TCP)

Test for biofilm formation for the *K. pneumoniae* isolates was performed as previously described (22,23) with some modifications. For this purpose, 96-well polystyrene microplates were used. Isolates were grown on nutrient agar for 18-24 hours at 37°C. One colony of each strain was ino-

culated in 5 ml of Brain Heart Infusion Broth (BHIB) and incubated at 37°C for 24 hours, then diluted to 1:100 in BHIB + 2% sucrose. Each well in the microplate was filled with 200µL of this dilution (three independent cultures for each species). A sterile broth of BHIB+2% sucrose was used as a negative control. The microplates were incubated at 37°C for 24 to 48 hours, after which the well contents were gently removed and rinsed four times with TBS (pH 7.2) and allowed to dry at 60°C for 30 min. Then, the cells adhering to the polystyrene support in the wells were coloured with 200µL of 1% (w/v) crystal violet for 30 minutes incubation at room temperature. The extra purple crystal violet was poured out and rinse four times at the sterile physiological waters. 200µL of ethanol 95% (v/v) was added to each well and left for 15 minutes before reading the optical density (OD) at 550nm using a microplate reader (Bio-Rad ELISA, PR 5100).

The interpretation of the results was carried out according to Vuotto et al., (15). The OD of the strains was obtained by the average of the three wells and compared to the OD (mean absorbance) of the negative control (OD<sub>c</sub>). Non-biofilm producer had OD<sub>c</sub> ≤ OD<sub>c</sub>, weak biofilm producer had OD<sub>c</sub> < OD ≤ 2xOD<sub>c</sub>, moderate biofilm producer had 2OD<sub>c</sub> < OD ≤ 4xOD<sub>c</sub>, and strong biofilm producer had 4xOD<sub>c</sub> < OD.

#### Statistical analysis

Data were analysed and presented on Excel sheet as frequency distribution tables and simple graphs. The susceptibility data were analyzed and interpreted using the WHONet 5.6 antibiotic susceptibility surveillance software

Table 1: Primer sequences used for PCR detection of ESBL genes

Gene	Sequence	Sequences (5'--3')	Size of the fragments (pb)
CTX-M group 1	CTX-M - F	5'-AAA AAT CAC TGC GCC AGTTC	415
	CTX-M - R	5'-AGC TTA TTC ATC GCC ACG TT	
CTX-M group 2	CTX-M - F	5'-CGA CGC TAC CCC TGC TAT T	552
	CTX-M - R	5'-CCA GCG TCA GAT TTT TCA GG	
CTX-M group 9	CTX-M - F	5'-CAA AGA GAG TGC AACGGA TG	205
	CTX-M - R	5'-ATT GGA AAG CGT TCA TCA CC	
CTX-M group 8	CTX-M - F	5'-TCG CGT TAA GCG GAT GAT GC	666
	CTX-M - R	5'-GCA CGA TGA CAT TCG GG	
CTX-M group 25	CTX-M - F	5'-GCA CGA TGA CAT TCG GG	327
CTX-M group 8/25	CTX-M - R	5'-AAC CCA CGA TGT GGG TAG C	
TEM	TEM - F	5'-ATG AGT ATT CAA CAT TTC CGT G	861
	TEM - R	5'-TTA CCA ATG CTT CAG TGA G AAT	

F = Forward; R= Reverse; bp = base pair

## Results:

During the study period, a total of 630 samples from 630 patients with clinical infections were collected and processed for isolation and identification of bacteria in the laboratory. *K. pneumoniae* was isolated in 40 (6.3%) samples. In the AST assay, all the *K. pneumoniae* isolates were resistant (100%) to ampicillin and ticarcillin, 10% to amoxicillin/clavulanic acid, and 2.5% to piperacillin/tazobactam. On the other hand, 100% of the isolates were sensitive to ceftazidime, imipenem and ertapenem, while sensitivity to other antibiotics were amikacin (97.5%), ofloxacin (87.5%), ciprofloxacin (82.5%), nalidixic acid (67.5%), gentamicin (67.5%), tobramycin (65%), cefotaxime (62.5%), ceftazidime (61.5%), cephalothin (60%), nitrofurantoin (55%), and trimethoprim/sulfamethoxazole (50%) (Fig 1).

The MIC of cefotaxime (CTX) was  $\geq 64\mu\text{g/ml}$ , MIC of ceftazidime (CAZ) ranged from 1- $\geq 64\mu\text{g/ml}$ , MIC of ceftazidime (FOX) was in the range of  $\leq 4-8\mu\text{g/ml}$  and MIC of amoxicillin/clavulanic acid (AMC) was between 16 and  $\geq 32\mu\text{g/ml}$ . Fifteen (15) of the 40 (37.5%) *K. pneumoniae* isolates were ESBL-producing strains (Fig 2a,b) but no carbapenemase producing strain was detected.

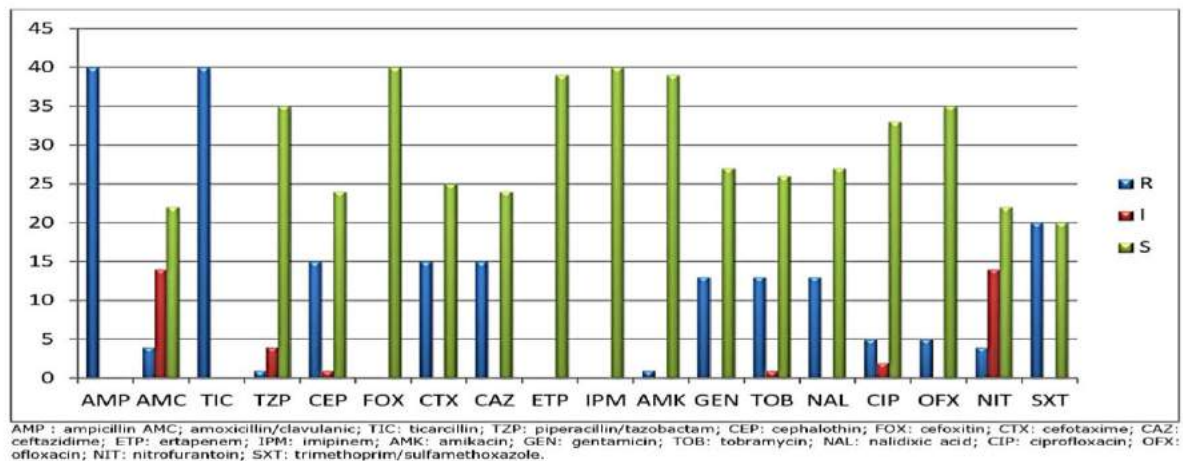


Fig 1: Antimicrobial susceptibility profiles of *Klebsiella pneumoniae* isolates

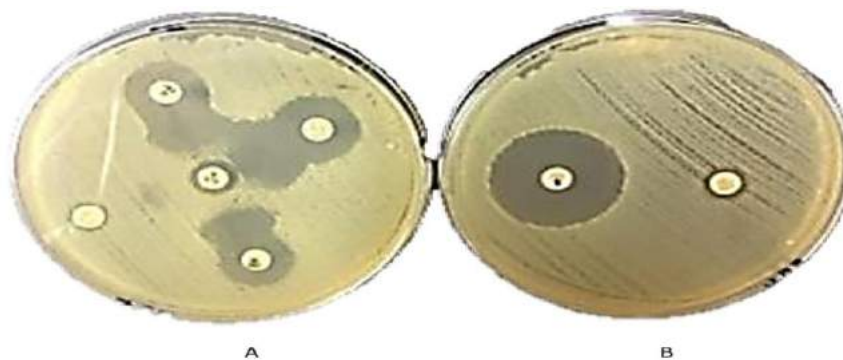


Fig 2a: ESBL-producing *Klebsiella pneumoniae*. A: test positive by synergy; B: test positive by combination disk

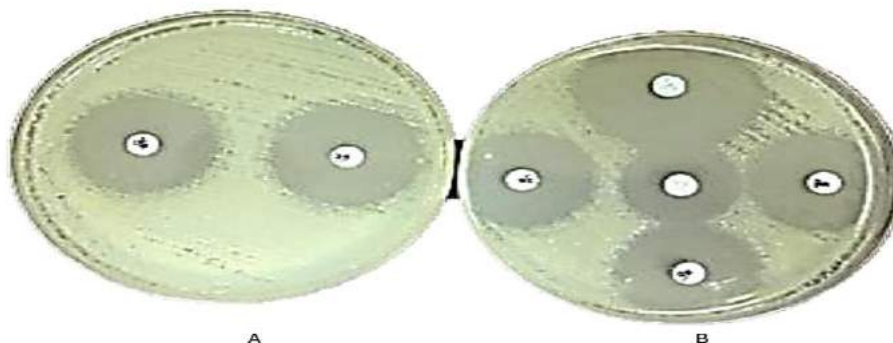


Fig 2b: Non-ESBL-producing *Klebsiella pneumoniae*. A: test negative by combination disk; B: test negative by synergy

The ES $\beta$ L-producing isolates were more frequently recovered from urine (46.7%), followed by pus, blood culture and perirectal abscess (13.3% each), and least from surgical wounds and catheters (6.7% each). There were no ES $\beta$ L-producing strains in the cerebrospinal fluids (Table 2).

Table 2: Distribution of *Klebsiella pneumoniae* and KP-ES $\beta$ L isolates according to patients' samples

Samples	Number positive for <i>K. pneumoniae</i> (%)	No positive for ES $\beta$ L (%)
Surgical wound (n=2)	1 (2.5)	1 (6.7)
Perirectal abscess (n=4)	2 (5.0)	2 (13.3)
Catheter urine (n=8)	2 (5.0)	1 (6.7)
Cerebrospinal fluid (n=12)	2 (5.0)	0
Blood (n=98)	2 (5.0)	2 (13.3)
Pus (n=173)	11 (27.5)	2 (13.3)
Urine (n=333)	20 (50.0)	7 (46.7)
Total (n=630)	40	15 (37.5)

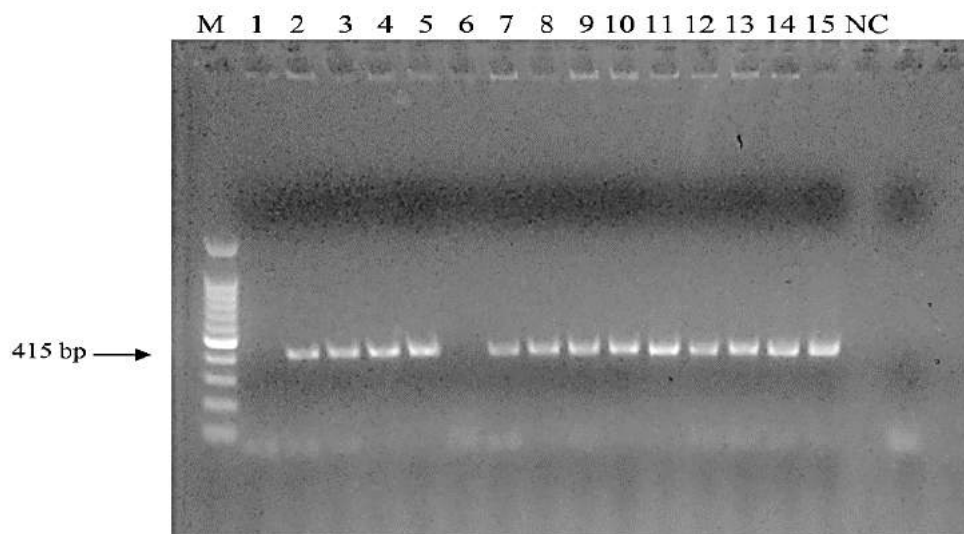
Twenty-seven *K. pneumoniae* isolates (65.7%) were recovered from male patients, 13 (48.2%) of which were ES $\beta$ L producers, while 13 isolates (32.5%) were recovered from female patients, 2 (15.4%) of which were ES $\beta$ L producers. The age group distribution of the patients with KP-ES $\beta$ L-producing isolates shows that 3 patients (20%) were in the age group 21-30 years, while 2 patients (13.3%) each were in age groups <10 years, 10-20 years, 31-40 years, 41-50

years, 51-60 years and >60 years. Most ES $\beta$ L-producing strains were isolated in the intensive care unit (33.3%, n=5), followed by general surgery (20%, n=3), paediatrics and urology units (13.3%, n=2) each, and 6.7% (n=1) each from nephrology, re-education and psychiatry units (Table 3).

Table 3: Distribution of *Klebsiella pneumoniae* and KP-ES $\beta$ L isolates by departments/units of patients

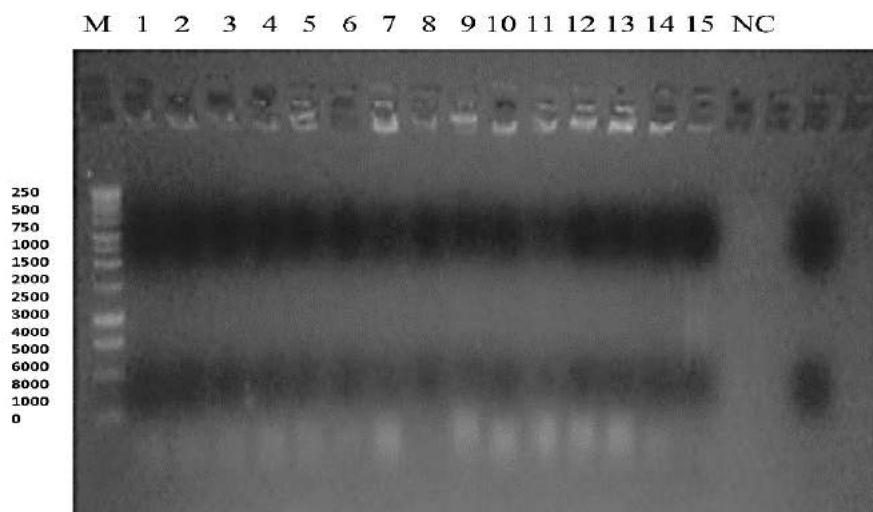
Service	Number of <i>K. pneumoniae</i> isolates	No positive for ES $\beta$ L (%)
Paediatrics	3	2 (13.3)
Infectious disease	6	0
General surgery	6	3 (20)
Oncology	1	0
Pneumology	3	0
Haematology	1	0
Intensive care unit	8	5 (33.3)
Urology	5	2 (13.3)
Re-education	1	1 (6.7)
Nephrology	4	1 (6.7)
Child surgery	1	0
Psychiatry	1	1 (6.7)
Total	40	15 (37.5)

Group 1 *bla*<sub>CTX-M</sub> gene was detected by PCR from 13 (86.7%) of the 15 KP-ES $\beta$ L producing isolates, while 2 (13.3%) isolates did not contain the gene (Fig 3a). No *bla*<sub>TEM</sub> gene was detected in any of the isolates (Fig 3b). The results of the biofilm formation with TCP test for KP-ES $\beta$ L producing isolates showed that 7 (46.7%) were moderate, 7 (46.7%) were weak biofilm producers, while 1 (6.7%) was non biofilm producer (Fig 4).



First lane, molecular weight marker; Gene Ruler 100 bp DNA ladder, lanes numbered 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15 show 415 bp bands of PCR products while lanes number 1 and 6 shows negative result. NC: Negative control

Fig 3a: Gel electrophoresis of the PCR products of *bla*<sub>CTX-M</sub> gene



First lane, molecular weight marker; Gene Ruler 100 bp DNA ladder; lanes number 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 shows negative result. NC: Negative control

Fig 3b: Gel electrophoresis of the PCR products of *bla*<sub>TEM</sub> gene

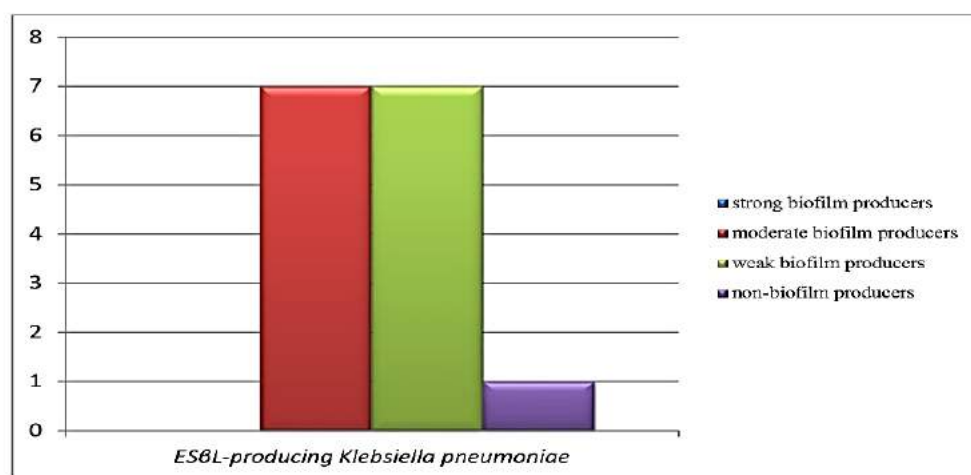


Fig 4: Detection of biofilm formation by the TCP method

## Discussion:

During the study period, *K. pneumoniae* was involved in 6.3% of clinical infections among the 630 patients, a rate that is similar to that reported by Khalifa and Khedher (12). All the *K. pneumoniae* isolates were resistant to ampicillin and ticarcillin (100%), which is consistent with the findings of Lagha et al., (24) and Muggeo et al., (25) in their previous studies. However, they were highly sensitive to amoxicillin/clavulanic acid (AMC) (90%) and piperacillin/tazobactam (97.5%), a finding that is also consistent with that of Rasamiravaka et al., (26) especially for AMC, although Muggeo et al., (25) and Khalifa and Khedher (12) reported *K. pneumoniae* isolates with intermediate resistance to AMC in their studies, while higher AMC resistance rates were reported by Lagha et al., in 2014 (24) and Benaicha et al., in 2017 (27). However, for piperacillin/tazobactam combination, the resistance rate (2.5%) in our study is lower

than the rates reported by Lagha et al., (24) and Muggeo et al., (25). The decrease in the resistance rate may be an indicator of the presence of CTX-M type  $\beta$ -lactamases and the association of penicillinases with inhibitors, which permit recovery of the activity of the molecules. The  $\beta$ -lactamase inhibitors have structural similarity with penicillin, and are effective against many sensitive organisms expressing class A lactamases (17). In addition, the combination of penicillin and  $\beta$ -lactamase inhibitor has been actively used in the treatment of infections caused by ESBL-producing bacteria and could be proposed for outpatient treatment of urinary tract infections caused by ESBL-producing *Escherichia coli* strains (1,28).

All the *K. pneumoniae* isolates in the study were sensitive to ceftazidime, imipenem and ertapenem which agrees with the reports of Alibi et al., (20) and Lagha et al., (24), al-

though different susceptibility rates have been reported in many other studies (11, 15,25,26,29). Currently, carbapenems are the only class of antimicrobials historically effective against KP-ES $\beta$ L producing strains (13), however, it is essential to ensure the rational use of carbapenems because there are no new antibiotics in the pipeline available for use in the near future for the treatment of infections caused by ES $\beta$ L-producing Enterobacteriaceae (1). On the other hand, *K. pneumoniae* isolates in our study were highly sensitive to amikacin, ofloxacin and ciprofloxacin, and moderately sensitive to cephalothin, cefotaxime, ceftazidime, gentamicin, tobramycin, and nalidixic acid, which agrees with findings of some other studies (15,17,24,27,29).

However, Muggeo et al., (25) and Rasamiravaka et al., (26) reported lower sensitivity (83%) to amikacin in their studies compared to 97.5% in our own study. Only half of the *K. pneumoniae* isolates were sensitive to trimethoprim/sulfamethoxazole and nitrofurantoin. Rasamiravaka et al., (26) and Vuotto et al., (15) have reported differing susceptibility rates of *K. pneumoniae* isolates to trimethoprim/sulfamethoxazole in their studies. The fluoroquinolones (FQ) are the potential drug of choice for treating infections caused by  $\beta$ -lactamase-producing enterobacteria that are usually FQ sensitive (25). However, Muggeo et al., (25) reported 100% resistance of *K. pneumoniae* ST395 to fluoroquinolones in north-eastern France, which is contrary to the findings of high susceptibility in our study. *Klebsiella* species are naturally sensitive to FQ, but the misuse of these antibiotic in human and veterinary medicine practices have, over the past decades, resulted in evolution of resistance to this antibiotic family, reduced their effectiveness, and compromised the future use of this important class of antibacterial drugs (12).

In our study, 15 of the 40 (37.5%) *K. pneumoniae* isolates were ES $\beta$ L-producing strains, which is proximate to 41.1% rate reported by Pirzaman et al., (2). Different prevalence rates for ES $\beta$ L-producing strains have been reported in Tunisia (12), Algeria (24) and Russia (30), and ES $\beta$ L rates are usually higher in Asian countries, with up to 75% (17). The phenomenon of ES $\beta$ L production has been observed in various pathogenic bacteria, but more frequent in *E. coli* and *K. pneumoniae*. The KP-ES $\beta$ L strains were most frequently recovered from urine in our study which is consistent with the findings of other studies (2,26,27,31,32,33), probably because urine is about the most commonly collected specimens for clinical investigation. Infections caused by ESBL-producing bacteria can occur in people of all ages, but distribution could be determined by the immunological status of

patients and prevalence of antibiotic misuse. KP-ES $\beta$ L isolates were recovered across all age groups in our study but most frequently in age group 21-30 years (20%), although the number of isolates from patients in our study are too few to make any significant inference. However, Lagha et al., (24) reported that the most KP-ES $\beta$ L were recovered from patients between the ages 27-85 years, Gravey et al., (34) reported 4.1% KP-ES $\beta$ L rate in the age group 18-64 years and 4.2% in the age group >65 years. *K. pneumoniae* isolates as well as KP-ES $\beta$ L strains were mostly recovered from male patients in our study. This is in close agreement with those of Bush et al., (33), Deng et al., (35) and Lagha et al., (24).

Also, most KP-ES $\beta$ L strains were isolated from the intensive care and general surgery units of the hospital, with 33.3% and 20% rates respectively. This agrees with the finding of Lagha et al., (24), but Khalifa and Khedher (12) reported lower rates than ours, with rates of 5% in paediatrics, 2.5% in medical resuscitation, and 0% in surgical unit. Numerous studies have reported isolation of KP-ES $\beta$ L strains from hospitalized patients and nosocomial epidemics caused by these strains have been reported mainly in intensive care units (20). We did not isolate KP-ES $\beta$ L strain from infectious, oncology, pneumology, haematology and child surgery departments of the hospital. Although, this might suggest good infection prevention and control practices in these service departments, the limited KP-ES $\beta$ L strains in this study cannot allow us to generalize this finding.

PCR assays detected group 1 *bla*<sub>CTX-M</sub> genes in most (86.7%) of the KP-ES $\beta$ L producing isolates, while few (13.3%) isolates did not carry the gene. This is similar to what Alibi et al., (20) reported in their study for *bla*<sub>CTX-M</sub> but in addition, they reported *bla*<sub>TEM</sub> in 56.8% of their isolates. However, Paterson et al., (36) reported 23.3% *bla*<sub>CTX-M</sub> and 87% *bla*<sub>TEM</sub> among their isolates. In Abidjan, Côte d'Ivoire, Guessennd et al., (31) reported that 63.4% of their strains carried *bla*<sub>TEM</sub>, 58.5% carried group 1 *bla*<sub>CTX-M</sub>, none carried groups 2 and 9 *bla*<sub>CTX-M</sub>, one strain carried group 8 *bla*<sub>CTX-M</sub> and three strains carried *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and group1 *bla*<sub>CTX-M</sub>. In another study in Korea by Jung et al., (32), only group 1 *bla*<sub>CTX-M</sub> (75.9%) and/or group 9 *bla*<sub>CTX-M</sub> (20.5%) were reported. In an Egyptian study, *bla*<sub>CTX-M</sub> was reported in three cases and *bla*<sub>TEM</sub> was detected only in one case (37). KP-ES $\beta$ L strains carrying *bla*<sub>CTX-M</sub> and/or *bla*<sub>TEM</sub> genes are usually resistant to third generation cephalosporins (ceftriaxone, cefotaxime, ceftazidime), and several studies have shown that the presence of these genes could confer resistance to the third-generation cephalosporins (38). With the wide-



spread use of cefotaxime and ceftriaxone (36), it is not surprising that *bla*<sub>CTX-M</sub> ESBL strains are now found in many countries and reported at high rates among *K. pneumoniae* and other bacteria pathogens (20).

It has been widely reported in the literature that biofilm production provides a significant benefit by protecting pathogens against host immune system and reducing susceptibility to antibiotic therapy. In our study, 46.7% of KP-ESBL strains were moderate and 46.7% were mild biofilm producers, which agrees with the finding of Seifi et al., (7). Martino et al., (39) reported 48.5% of *K. pneumoniae* strains to be strong biofilm producers; Vuotto et al., (15) reported 67.5% of strains to be potent, and 25% to be moderate biofilm producers, and Khodadadian et al., (11) reported 91.2% of their isolates to be biofilm producers. Surgers et al., (40) have reported a close relationship between several virulence factors and the ability to produce biofilm. The ability of *K. pneumoniae* strains to adhere and colonize inert surfaces may be a general feature of this species, as high incidence of effective adhesion of *K. pneumoniae* strains to glass and polypropylene surfaces in clinical and water distribution systems, have been observed (39).

## Conclusion:

Infections caused by ESBL-producing Gram-negative bacteria are increasing, particularly in immunocompromised patients and in high-risk units of hospitals. These infections are associated with higher costs of healthcare in Algeria and worldwide, as a result of prolonged hospitalization and the use of expensive drugs. The prevalence of KP-ESBL-producing strains reported in this study is a reflection of the level of infection prevention and control practices in our hospital.

Surveillance of these antibiotic resistant pathogens by detection of  $\beta$ -lactamases and molecular identification of prevalent ESBL genes, as well as good knowledge of biofilms formation, will be essential for reliable epidemiological characterization of these pathogens, in order to prevent the risk of transmission, and implement antibiotic stewardship and appropriate infection control measures.

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Copyright AJCEM 2021: <https://dx.doi.org/10.4314/ajcem.v22i1.6>**Original Article****Open Access****ESBL, AmpC and carbapenemase co-production in multi-drug resistant Gram-negative bacteria from HIV-infected patients in southwestern Nigeria**

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Department of Microbiology, Faculty of Basic and Applied Sciences,  
Osun State University, Osogbo, Nigeria\*Correspondence to: [folasade.adeyemi@uniosun.edu.ng](mailto:folasade.adeyemi@uniosun.edu.ng); +234 803 494 0747**Abstract:**

**Background:** The rising global emergence of Gram-negative bacteria (GNB) producing  $\beta$ -lactam hydrolysing enzymes in clinical infections constitutes a growing public health threat. This study investigated the occurrence of co-production of extended spectrum  $\beta$ -lactamase (ESBL), AmpC  $\beta$ -lactamases, and carbapenemases among GNB isolated from HIV-infected patients in two tertiary healthcare facilities in southwest Nigeria.

**Methodology:** A total of 115 GNB isolates previously recovered from HIV-infected patients at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, and the State Specialist Hospital, Akure, were investigated. The isolates were characterized to species level with the Microbact 24E kit and screened for ESBL production using the double-disc test (DDT) and combination disc methods, AmpC using modified Hodge test (MHT) and AmpC EDTA disc, and carbapenemase production using the MHT and EDTA disc test. Antibiotic susceptibility testing (AST) was performed by the Kirby-Bauer disc diffusion method.

**Results:** A total of 15 species of GNB were characterized. The AST profile of the isolates revealed high resistance rates to ampicillin (94.5%), tetracycline (74.5%), sulphamethoxazole-trimethoprim (66.3%), and lowest resistance to imipenem (10.9%). Multi-drug resistance (MDR) was observed in 93.6% while 98.8% of ESBL, AmpC, and carbapenemase-producing isolates had multiple antibiotic resistance (MAR) indices  $\geq 0.2$ . ESBL production was detected in 53.9%, AmpC in 20.9% and carbapenemase in 25.2% of the isolates. ESBL, AmpC or carbapenemase or co-production of two or all three enzymes was detected in 80 (69.6%) isolates, while only 10.0% produced all three enzymes.

**Conclusion:** The isolation of MDR bacteria and isolates co-producing  $\beta$ -lactam hydrolysing enzymes in immunocompromised individuals portend grave consequences. Routine screening for these enzymes in MDR bacteria will be highly essential to guide the institution of appropriate antibiotic therapy and infection control measures.

**Keywords:** ESBL, AmpC, carbapenemase, HIV, MDR, clinical isolates, MHT, DDST

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**Coproduction d'ESBL, AmpC et carbapénémase dans des bactéries Gram-négatives multirésistantes de patients infectés par le VIH dans le sud-ouest du Nigéria**

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Université d'État d'Osun, Osogbo, Nigéria\*Correspondance avec: [folasade.adeyemi@uniosun.edu.ng](mailto:folasade.adeyemi@uniosun.edu.ng); +234 803 494 0747**Abstrait:**

**Contexte:** L'émergence mondiale croissante de bactéries à Gram négatif (GNB) produisant des enzymes d'hydrolyse de  $\beta$ -lactame dans les infections cliniques constitue une menace croissante pour la santé publique. Cette étude a examiné l'occurrence de la coproduction de  $\beta$ -lactamases à spectre étendu (ESBL), de  $\beta$ -lactamases AmpC et de

carbapénémases parmi les GNB isolés de patients infectés par le VIH dans deux établissements de santé tertiaires du sud-ouest du Nigéria.

**Méthodologie:** Un total de 115 isolats de GNB précédemment récupérés de patients infectés par le VIH au complexe hospitalier universitaire Obafemi Awolowo, Ile-Ife, et au State Specialist Hospital, Akure, ont été étudiés. Les isolats ont été caractérisés au niveau des espèces avec le kit Microbact 24E et criblés pour la production d'ESBL en utilisant le test à double disque (DDT) et les méthodes de disques combinés, AmpC en utilisant le test Hodge modifié (MHT) et le disque AmpC EDTA, et la production de carbapénémase en utilisant le MHT et test de disque EDTA. Le test de sensibilité aux antibiotiques (AST) a été effectué par la méthode de diffusion de disque de Kirby-Bauer.

**Résultats:** Un total de 15 espèces de GNB ont été caractérisées. Le profil AST des isolats a révélé des taux de résistance élevés à l'ampicilline (94,5%), à la tétracycline (74,5%), au sulfaméthoxazole-triméthoprim (66,3%) et à la plus faible résistance à l'imipénème (10,9%). Une résistance à plusieurs médicaments (MDR) a été observée dans 93,6% tandis que 98,8% des isolats producteurs d'ESBL, AmpC et carbapénémase avaient de multiples indices de résistance aux antibiotiques (MAR)  $\geq 0,2$ . La production d'ESBL a été détectée dans 53,9%, AmpC dans 20,9% et carbapénémase dans 25,2% des isolats. ESBL, AmpC ou carbapénémase ou la coproduction de deux ou des trois enzymes a été détectée dans 80 isolats (69,6%), tandis que seulement 10,0% ont produit les trois enzymes.

**Conclusion:** L'isolement des bactéries MDR et des isolats co-producteurs d'enzymes d'hydrolyse des  $\beta$ -lactamines chez les individus immunodéprimés laisse présager de graves conséquences. Le dépistage systématique de ces enzymes dans les bactéries MDR sera très essentiel pour guider la mise en place d'une antibiothérapie appropriée et de mesures de contrôle des infections.

**Mots-clés:** ESBL, AmpC, carbapénémase, VIH, MDR, isolats cliniques, MHT, DDST

## Introduction:

The use of antibiotics for the treatment of infections in humans and livestock usually result in the selection of pathogenic bacteria which become multiply resistant to them (1,2). Several strains of Gram-negative bacteria (GNB) are becoming resistant to practically all the commonly available antibacterial agents (3,4). Prominent among these GNB are the extended-spectrum  $\beta$ -lactamase (ESBL) enterobacteria (5).

ESBLs are extended-spectrum  $\beta$ -lactamases that are capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, as well as to aztreonam except cephamycins or carbapenems (6-9). ESBLs are mutants of TEM-1, TEM-2, and SHV-1 which act by hydrolysing  $\beta$ -lactam antibiotics but are inhibited by  $\beta$ -lactamase inhibitors such as clavulanic acid (10), sulbactam, and tazobactam (11). However, the CTX-M type ESBLs have become more broadly distributed and globally prevalent (12-14).

AmpC  $\beta$ -lactamases are clinically significant because they may confer resistance to penicillins, cephalosporins (15), oxyimino-cephalosporins, cephamycins (16), and monobactams. Unlike ESBL, AmpC  $\beta$ -lactamase activity is not affected by clavulanic acid (17). Although reported with increasing frequency in clinical isolates, the actual occurrence rate of plasmid-mediated AmpC  $\beta$ -lactamases in Enterobacteriaceae remains unknown as many laboratories have difficulty in detecting these enzymes in clinical isolates (18).

Carbapenems are powerful, broad-spectrum antibiotics, which are often considered to be the last line of defence in the treatment of infections caused by multidrug-resistant (MDR) GNB (19) because they are stable even in the

presence of the extended-spectrum and AmpC  $\beta$ -lactamases. Carbapenemases are ESBLs produced by resistant bacteria strains (20) which possess hydrolytic capacity against almost all  $\beta$ -lactam antibiotics including carbapenems, and constitute the most versatile family of  $\beta$ -lactamases belonging to molecular classes-A, B and D.

ESBL and carbapenemase producing bacteria have become significant threats to patients in the hospital (21) and long-term care facilities as well as to immuno-compromised persons in the community. Worldwide, there is a rapid increase in the prevalence of ESBL, AmpC, and carbapenemase producing members of the family Enterobacteriaceae (21-27), and infections caused by them are associated with increased morbidity, mortality, and health care costs (21,28-31). Significant increase in the incidence of ESBL-related infections has been reported by various authors from around the world (32-36).

The increasing use of carbapenems to treat infections caused by ESBL-producing GNB is creating a ripple effect by causing increase in the prevalence of carbapenemase-producing bacteria. The screening and probable detection of ESBL production among bacteria isolates from immuno-compromised individuals as the case with HIV-infected patients, is of tremendous clinical significance because this may be an epidemiologic marker of colonisation (37), which may invariably portend severe implications in this group of patients. Again, these organisms exhibit co-resistance to many other classes of antibiotics because plasmids that encode ESBL genes most frequently carry genes encoding resistance to other drug classes, leading to failure in the treatment regimen and making therapeutic options limited (38).

There are various studies on the prevalence of ESBL producing bacteria that have been conducted in different parts of Nigeria (7,39-46) with rates varying between 7.5% (43,47) and 58.6% (42), depending on the samples from which the isolates were obtained. A systematic review by Manenzhe et al., (48) reported that carbapenemase producers occur widely in Africa and have been reported in Nigeria (48). Le Terrier et al., (49) also recently reported the preponderance of carbapenemase-producers in a Nigerian environment. Because of this, routine monitoring of clinical isolates for these traits with particular emphasis on patients who are immunocompromised is important. In this study, we evaluated the production of ESBL, AmpC, and carbapenemase among selected GNB isolates recovered from HIV-infected patients in two tertiary healthcare facilities in southwestern Nigeria using two phenotypic screening methods for the detection of each enzyme.

## Materials and method:

### Gram-negative isolates from participants:

The GNB isolates (n=115) investigated in this study were randomly selected from a collection of 316 GNB cultured from skin, throat, and rectal swabs of HIV seropositive patients in our previous study (50,51). The patients were recruited from two hospitals; Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, and State Specialist Hospital, Akure, Ondo State, over a period of 18 months. Where two isolates from one patient were included, the strains were unique regarding species identification or resistance pattern.

A preformed questionnaire indicating participant demographic information including age, gender, and other relevant information was used to collate data. Approval for the study was obtained from the Ethical Review Board of the Obafemi Awolowo University Teaching Hospital, Ile-Ife and the Management Board of Ondo State Specialist Hospital, Akure.

### Identification of the GNB isolates

The isolates, preserved in TSB with 15% glycerol broth at -20°C, were sub-cultured and purified on MacConkey agar (Oxoid, UK) for identification to species level. The inoculum was prepared by picking one or two well-isolated colonies and emulsifying in 5ml Ringer's solution (Oxoid, UK). The Microbact™ GNB 24E system was inoculated according to the manufacturer's instructions and incubated at 35±2°C for 18–24 hours for oxidase-negative strains and 48 hours for oxidase-positive strains of the GNB. The wells were observed for colour change following

the addition of appropriate reagents in line with the manufacturer's instruction. Each well was ascribed a numerical value indicating either a positive or negative reaction which was converted to a 9-digit code for final identification with the Microbact™ identification software version 2.04. The acceptable species identification was ≥ 80.0% and ≤ 99.9%.

### Antibiotic susceptibility testing of GNB isolates

An *in vitro* antibiotic susceptibility assay was carried out on 110 of the 115 GNB isolates using the Kirby-Bauer disc diffusion method. Eleven antibiotics (Oxoid, UK) selected based on their frequent use in research and clinical therapy included ampicillin (10µg), chloramphenicol (30µg), colistin (10µg), gentamicin (10 µg), imipenem (10µg), nalidixic acid (30µg), nitrofurantoin (300 µg), piperacillin (30 µg), piperacillin/tazobactam (36µg), tetracycline (30 µg) and trimethoprim/sulfamethoxazole (25µg).

Quality control was performed using *Enterobacter aerogenes* ATCC 13048 to confirm the consistency of materials, methods, and results. Interpretation of results as either susceptible, intermediate, or resistant was done using the zone diameter breakpoints recommended by Clinical and Laboratory Standards Institute (52). MDR pattern among the GNB isolates was defined as resistance to ≥ 1 agent in ≥ 3 antibiotic classes (53). Multiple antibiotic resistance (MAR) index for each isolate was evaluated by dividing the number of antibiotics to which isolate was resistant by the total number of antibiotics against which the bacteria isolate was tested (54).

### Detection of ESBL, AmpC, and carbapenemase in GNB isolates

#### Phenotypic ESBL screening:

Screening for ESBL production was done using two methods: the double-disc synergy test (DDST) and the combination disc test methods. DDST was carried out, as described by Jarlier et al., (55). Using a sterile cotton-tipped applicator, Mueller-Hinton agar was inoculated with standardised test organism (0.5 McFarland turbidity standard) in Ringer's solution to give a semi-confluent growth. Discs containing 30µg each of aztreonam, ceftriaxone, cefotaxime, and ceftazidime, were placed 30mm apart (centre to centre) around a disc containing amoxicillin (20 µg) plus clavulanic acid (10µg) on the agar surface, incubated at 35±2°C for 18-24 hours. Inhibition zone enhancement which indicates synergy between clavulanic acid and any one of test antibiotics, was regarded as presumptive ESBL production. *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 strains

served as positive and negative control strains respectively.

The combination disc test was performed using disc pairs containing cephalosporin (cefotaxime, ceftazidime, and cefpodoxime) with and without clavulanic acid in each case (56). Inoculation was carried out as described for DDST. The paired discs were placed on the inoculated plates with a distance of at least 25mm separating them. The zones of inhibition were measured following overnight incubation at  $35\pm 2^{\circ}\text{C}$ . Inhibition zone of  $\geq 5\text{mm}$  or expansion by 50% around the combination disc (cephalosporin with clavulanic acid) compared to the cephalosporin disc alone was indicative of ESBL production (56).

#### **AmpC $\beta$ -lactamase detection:**

Screening for AmpC  $\beta$ -lactamase production was done by the AmpC disc test and modified Hodge test (MHT). AmpC disc test was carried out as described by Black et al., (57) with modification. AmpC discs were prepared in-house by applying  $20\mu\text{l}$  of a 0.5 M Tris-EDTA to sterile filter paper discs and dried, then rehydrated with  $20\mu\text{l}$  saline just before use. Cefoxitin susceptible *Escherichia coli* ATCC 8739 was used for lawn preparation on Mueller-Hinton agar. Cefoxitin disc ( $30\mu\text{g}$ ) was placed on the inoculated media next to AmpC disc inoculated with the test isolate, with the inoculated disc face in contact with the agar surface. After overnight incubation at  $35\pm 2^{\circ}\text{C}$ , an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin was recorded as a positive result, while the absence of a distortion was recorded as a negative result.

For the MHT test, a suspension of the cefoxitin susceptible strain of *E. coli* ATCC 8739 was prepared, diluted 1:10 with physiological saline, and swabbed across a Mueller Hinton plate with a sterile cotton-tipped applicator. A  $30\mu\text{g}$  cefoxitin disc was placed at the centre of the test plate, and the test isolate was streaked in a straight line from the edge of the disc towards the edge of the plate. The plate was incubated overnight at  $35\pm 2^{\circ}\text{C}$ . A positive test was indicated by the growth of the *E. coli* ATCC 8739 along the line of streak of the test isolate towards the cefoxitin disc, while there was no growth of the *E. coli* on the line of streak in a negative result.

#### **Carbapenemase production:**

The phenotypic screening for carbapenemase production was carried out using the MHT and the EDTA disc methods. The MHT was

as described for  $10\mu\text{g}$  imipenem but opposed to cefoxitin for AmpC screening. The *E. coli* strain ATCC 8739 used was also susceptible to imipenem. A positive test was indicated by a clover leaf-like indentation of the *E. coli* 8739 growing along the growth streak of the test organism within the disc diffusion zone, while a negative test showed no growth of the *E. coli* 8739 along the test organism growth streak within the disc diffusion zone. Quality control of the carbapenem discs was performed according to CLSI guidelines (52) by running MHT positive *Klebsiella pneumoniae* ATCC 1705 and MHT negative *Klebsiella pneumoniae* ATCC 1706 with each batch of the test.

For the EDTA disc test, a 0.5M EDTA solution was first prepared by dissolving 9.36g of EDTA and 6.05g of TRIS base in 50ml of distilled water in a 50ml volumetric flask, and its pH was adjusted to 8.0 by using NaOH. A  $1\mu\text{l}$  of the 0.5M Tris-EDTA solutions was then dispensed onto sterile plain discs and the discs were used immediately. A lawn of the test isolate was plated on Mueller Hinton agar plate using a sterile cotton-tipped applicator and an imipenem disc ( $10\mu\text{g}$ ) was placed in the centre of the plate with sterile forceps. The Tris-EDTA discs inoculated with colonies of the test strain were placed about 1mm to the imipenem disc with the inoculated side touching the agar. The plates were incubated at  $35\pm 2^{\circ}\text{C}$  in ambient air for 16–24 hours. Indentation of the inhibition zone around the inoculated disc was indicative of the production of carbapenem-hydrolysing enzyme (positive test).

#### **Data analysis**

Data on identification, susceptibility and enzyme production of the GNB isolates were analysed using the Statistical Package for the Social Sciences (SPSS) version 23.0, and results presented with frequency distribution tables and simple graphs.

#### **Results:**

The identification and distribution pattern of the 115 GNB bacteria isolates randomly selected for the study is shown in Table 1, with 80 (69.6%) oxidase-positive and 35 (30.4%) oxidase-negative. The GNB isolates were characterized into 15 species (5 species for oxidase-positive and 10 species for oxidase-negative). A total of 80 (69.6%) isolates produced ESBL, AmpC, carbapenemase, or co-produced either two or three of the enzymes.

Table 1. Distribution of selected Gram-negative isolates from HIV seropositive patients

Isolate identity	Frequency distribution [number (%)]			
	Isolates from Skin	Isolates from Throat	Isolates from Rectal Swabs	Total isolates
<u>Oxidase Positive</u>				
<i>Actinobacillus</i> sp.	1 (1.2)	0 (0.0)	1 (4.5)	2 (1.7)
<i>Pseudomonas fluorescens</i> 25	6 (7.3)	0 (0.0)	0 (0.0)	6 (5.2)
<i>Burkholderia pseudomallei</i>	10 (12.2)	1 (9.1)	6 (27.3)	17 (14.8)
<i>Aeromonas hydrophila</i>	17 (20.7)	1 (9.1)	3 (13.6)	21 (18.2)
<i>Burkholderia cepacia</i>	24 (29.3)	4 (36.3)	6 (27.3)	34 (29.5)
<u>Oxidase Negative</u>				
<i>Citrobacter freundii</i>	1 (1.2)	0 (0.0)	0 (0.0)	1 (0.9)
<i>Citrobacter youngae</i>	1 (1.2)	0 (0.0)	0 (0.0)	1 (0.9)
<i>Morganella morganii</i> biogp 1	1 (1.2)	0 (0.0)	0 (0.0)	1 (0.9)
<i>Photobacterium asymbiotica</i>	1 (1.2)	0 (0.0)	0 (0.0)	1 (0.9)
<i>Pragia fontium</i>	1 (1.2)	0 (0.0)	0 (0.0)	1 (0.9)
<i>Serratia marcescens</i>	1 (1.2)	0 (0.0)	0 (0.0)	1 (0.9)
<i>Proteus vulgaris</i>	2 (2.4)	1 (9.1)	0 (0.0)	3 (2.6)
<i>Photobacterium luminescens</i> 25C	1 (1.2)	1 (9.1)	1 (4.5)	3 (2.6)
<i>Proteus mirabilis</i>	7 (8.5)	1 (9.1)	2 (9.1)	10 (8.7)
<i>Pantoea agglomerans</i> complex	8 (9.8)	2 (18.2)	3 (13.6)	13 (11.3)
Total	82 (71.3)	11 (9.6)	22 (19.1)	115

#### Antibiotic resistance profiles of GNB isolates

Out of the 115 GNB isolates, AST was performed only on 110. The AST profile of the isolates revealed that 94.5% (104/110) were resistant to ampicillin, 74.5% to tetracycline, and 66.3% to sulfamethoxazole-trimethoprim. The isolates exhibited lowest resistance rate to imipenem at 10.9% (12/110), closely followed by piperacillin/tazobactam at 13.6% and gentamicin at 15.5% (Table 2).

For the 80 enzyme-producing isolates, the least resistance rate was observed with piperacillin/tazobactam (11.3%), followed by imipenem (13.8%) and gentamicin (17.5%) while the highest resistance was to ampicillin, tetracycline, and sulfamethoxazole-trimethoprim at 93.8%, 72.5%, and 70.0% respectively (Table 3).

All the isolates were resistant to at least one antibiotic and none was sensitive to all the antibiotics. Only 1 (0.9%) of the isolates tested was resistant to only one antibiotic and 6 (5.4%) to only two antibiotics. This implied a high level of MDR as 93.6% (103/110) were resistant to more than three classes of antibiotics (Fig 1).

For ESBL, AmpC, and carbapenemase-producing strains, 82.5% (66/80) isolates were resistant to  $\geq 4$  classes of antibiotics. Only one isolate (1.25%), *Aeromonas hydrophila* was resistant to one antibiotic class (ampicillin), 6 isolates (7.5%) to two classes, and 7 isolates (8.75%) to three antibiotic classes. The multiple antibiotic resistance index (MAR index) (Fig 2) showed that 97.3% of the total number of isolates and 98.8% of ESBL, AmpC, and carbapenemase-producing strains had MAR indices  $\geq 0.2$ .

#### Co-production of ESBL, AmpC, and carbapenemase in GNB isolates

##### Pattern of ESBL production

Using either one or both ESBL screening methods, 53.9% (62/115) of the GNB were ESBL producers; 32 (27.8%) were detected by the combination disc method (10 *Burkholderia cepacia*, 7 *Aeromonas hydrophila*, 5 *Burkholderia pseudomallei*, 5 *Pantoea agglomerans* complex, 3 *Proteus mirabilis*, and 1 each of *Pseudomonas fluorescens* 25 and *Serratia marcescens*)

Table 2: Antibiotic resistance profile of selected Gram-negative isolates from HIV seropositive patients

Isolate identity	Number tested	Resistance profile (number of isolates)										
		AMP	PIP	PTZ	IPM	GEN	CHL	NA	NFT	TET	COL	SXT
<u>Oxidase Positive</u>												
<i>Actinobacillus</i> sp.	2	2	0	0	0	1	0	1	1	1	1	1
<i>Pseudomonas fluorescens</i> 25	6	5	4	2	0	0	3	2	5	3	0	3
<i>Burkholderia pseudomallei</i>	16	16	6	0	1	3	10	4	8	13	7	12
<i>Aeromonas hydrophila</i>	20	18	13	5	2	3	10	8	10	15	5	15
<i>Burkholderia cepacia</i>	34	33	19	5	4	4	18	12	23	24	12	20
<u>Oxidase Negative</u>												
<i>Citrobacter freundii</i>	1	1	1	0	0	0	1	1	1	1	0	1
<i>Citrobacter youngae</i>	1	1	1	0	0	0	0	1	1	1	0	1
<i>Morganella morganii</i> biogp 1	1	1	0	0	0	0	1	0	1	1	1	0
<i>Photorhabdus asymbiotica</i>	1	1	1	0	1	0	1	0	1	1	1	1
<i>Pragia fontium</i>	1	1	1	0	0	0	1	0	1	1	1	1
<i>Serratia marcescens</i>	1	1	0	0	0	0	1	1	0	1	0	1
<i>Proteus vulgaris</i>	3	3	0	0	0	1	3	1	2	2	2	2
<i>Photorhabdus luminescens</i> 25C	3	3	2	0	0	0	2	0	2	2	2	1
<i>Proteus mirabilis</i>	9	8	3	0	2	1	6	2	6	8	4	6
<i>Pantoea agglomerans</i> complex	11	10	4	3	2	4	7	2	4	8	6	8
TOTAL*	110	104	55	15	12	17	64	35	66	82	42	73
(%)		(94.5)	(50.0)	(13.6)	(10.9)	(15.5)	(55.1)	(31.8)	(60.0)	(74.5)	(38.1)	(66.3)

\*Values represent number of isolates (%). AMP - Ampicillin (10 µg); PIP - Piperacillin (30 µg); PTZ - Piperacillin/ Tazobactam (36 µg); IPM - Imipenem (10 µg); GEN - Gentamicin (10 µg); CHL - Chloramphenicol (30 µg); NA - Nalidixic acid (30 µg); NFT - Nitrofurantoin (300 µg); TET - Tetracycline (30 µg); COL - Colistin (10 µg); SXT - Trimethoprim/Sulfamethoxazole (25 µg)

Table 3: Antibiotic resistance profile of the enzyme producing Gram-negative isolates from HIV seropositive patients

Isolate Identity	No of producers	Resistance profile (number of isolates)										
		AMP	PIP	PTZ	IPM	GEN	CHL	NA	NFT	TET	COL	SXT
<u>Oxidase Positive</u>												
<i>Pseudomonas fluorescens</i> 25	4	3	3	2	0	0	1	2	3	2	0	2
<i>Burkholderia pseudomallei</i>	12	12	4	0	1	2	6	2	4	9	5	9
<i>Aeromonas hydrophila</i>	14	13	9	1	2	3	9	4	7	10	4	11
<i>Burkholderia cepacian</i>	25	24	15	3	3	4	14	10	15	17	11	16
<u>Oxidase Negative</u>												
<i>Citrobacter freundii</i>	1	1	1	0	0	0	1	1	1	1	0	1
<i>Citrobacter youngae</i>	1	1	1	0	0	0	0	1	1	1	0	1
<i>Morganella morganii</i> biogp 1	1	1	0	0	0	0	1	0	1	1	1	0
<i>Photorhabdus asymbiotica</i>	1	1	1	0	1	0	1	0	1	1	1	1
<i>Serratia marcescens</i>	1	1	0	0	0	0	1	1	0	1	0	1
<i>Photorhabdus luminescens</i> 25C	2	2	2	0	0	0	2	0	1	1	1	1
<i>Proteus mirabilis</i>	7	6	2	0	2	1	5	1	4	6	3	5
<i>Pantoea agglomerans</i> complex	11	10	4	3	2	4	7	2	4	8	6	8
TOTAL*	80	75	42	09	11	14	48	24	42	58	32	56
(%)		(93.8)	(52.5)	(11.3)	(13.8)	(17.5)	(60.0)	(30.0)	(52.5)	(72.5)	(40.0)	(70.0)

\*Values represent number of isolates (%). AMP - Ampicillin (10 µg); PIP - Piperacillin (30 µg); PTZ - Piperacillin/ Tazobactam (36 µg); IPM - Imipenem (10 µg); GEN - Gentamicin (10 µg); CHL - Chloramphenicol (30 µg); NA - Nalidixic acid (30 µg); NFT - Nitrofurantoin (300 µg); TET - Tetracycline (30 µg); COL - Colistin (10 µg); SXT - Trimethoprim/ Sulfamethoxazole (25 µg)



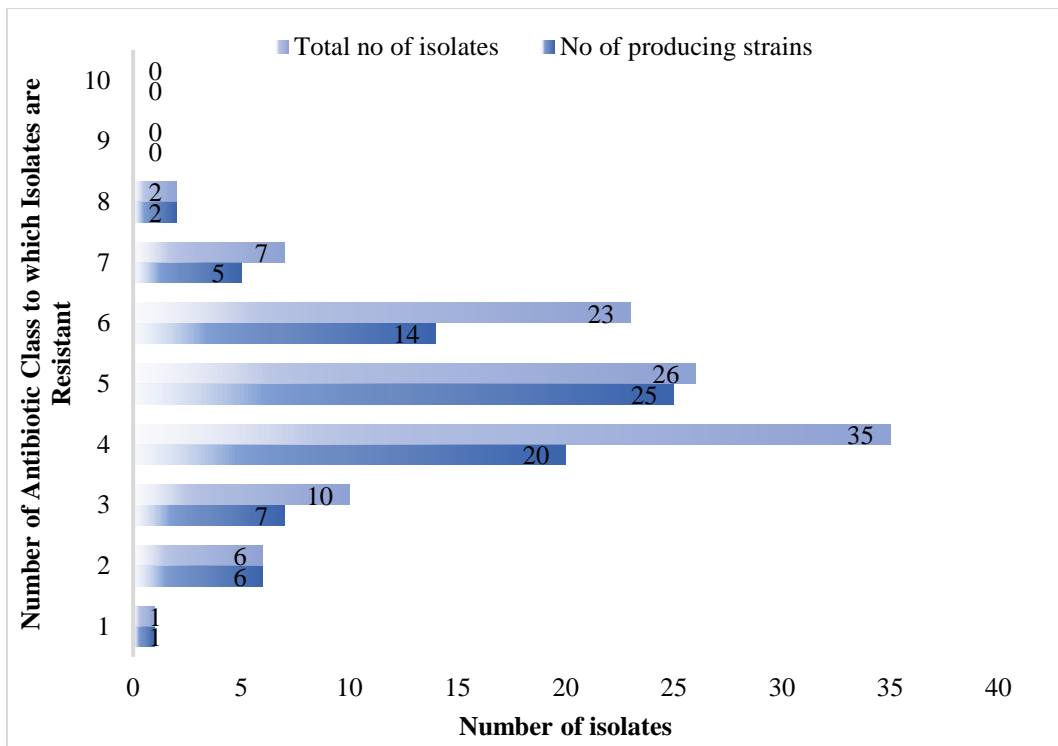


Fig 1: Multiple resistance profile of selected Gram-negative isolates from HIV seropositive patients

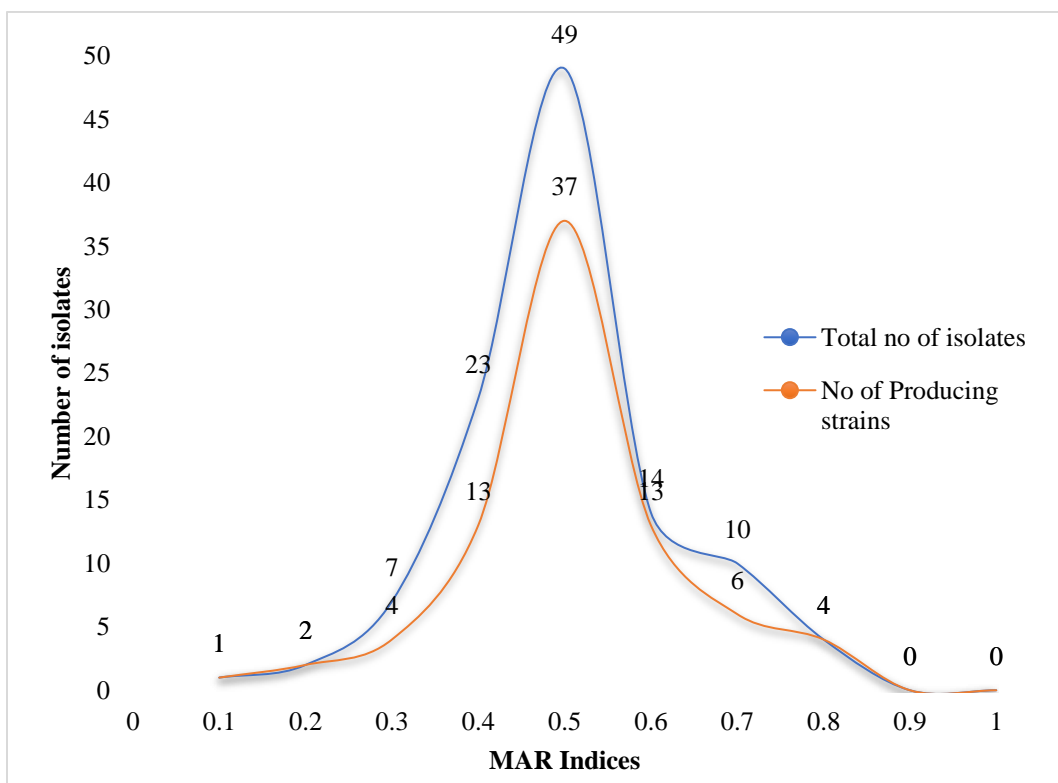


Fig 2: MAR index pattern of selected Gram-negative bacterial isolates from HIV seropositive patients

while 42 isolates were detected with the double-disc test (15 *Burkholderia cepacia*, 8 *Pantoea agglomerans* complex, 6 *Burkholderia pseudomallei*, 4 *Aeromonas hydrophila*, 3 each of *Proteus mirabilis* and *Pseudomonas fluorescens* 25, 2 *Photobacterium luminescens* 25C and 1 *Photobacterium asymbiotica*). However, ESBL production was detected in 12 (10.4%) GNB isolates using both phenotypic methods, and these were 5 *Burkholderia cepacia*, 3 *Pantoea agglomerans* complex, 2 *Burkholderia pseudomallei*, and 1 each of *Proteus mirabilis* and *Pseudomonas fluorescens* 25 (Table 4).

#### Pattern of AmpC $\beta$ -lactamase production

The results of the AmpC  $\beta$ -lactamase enzyme detection for all the 115 selected GNB isolates revealed that 20.9% (24/115) were positive for AmpC  $\beta$ -lactamase production; 15 with MHT alone (4 each of *Burkholderia cepacia*

and *Pantoea agglomerans* complex, 3 *Burkholderia pseudomallei*, 2 *Aeromonas hydrophila* and 1 each of *Photobacterium asymbiotica* and *Proteus mirabilis*), 5 with the AmpC EDTA disc test alone (2 *Burkholderia cepacia*, 1 each of *Aeromonas hydrophila*, *Burkholderia pseudomallei* and *Citrobacter freundii*), and 4 with both tests (2 *Pantoea agglomerans* complex, 1 *Burkholderia cepacia*, and 1 *Proteus mirabilis*) (Table 4).

#### Pattern of carbapenemase production

Twelve of the 15 GNB species screened for carbapenemase production had at least one positive isolate in a species except for *Actinobacillus* sp., *Pragia fontium*, and *Proteus vulgaris*. Altogether, 29 GNB isolates (25.2%) were positive for carbapenemase production; 4 with the modified Hodge test alone; 23 with the EDTA disc test alone, and 2 isolates with both phenotypic methods (Table 4).

Table 4: Detection of ESBL, AmpC and carbapenemase production in selected Gram-negative isolates from HIV seropositive patients

Isolate identity	Number of isolates tested	EsβL					AmpC				Carbapenemase			
		CDT	DDT	CDT/ DDT <sup>a</sup>	Total Positive	MHT	EDTA	MHT/ EDTA <sup>b</sup>	Total Positive	MHT	EDTA	MHT/ EDTA	Total Positive	
<u>Oxidase Positive</u>														
<i>Actinobacillus</i> sp.	2	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Pseudomonas fluorescens</i> 25	6	0	2	1	3	0	0	0	0	0	2	0	2	
<i>Burkholderia pseudomallei</i>	17	3	4	2	9	3	1	0	4	0	4	0	4	
<i>Aeromonas hydrophila</i>	21	7	4	0	11	2	1	0	3	1	3	0	4	
<i>Burkholderia cepacian</i>	34	5	10	5	20	4	2	1	7	0	7	0	7	
<u>Oxidase Negative</u>														
<i>Citrobacter freundii</i>	1	0	0	0	0	0	1	0	1	0	1	0	1	
<i>Citrobacter youngae</i>	1	0	0	0	0	0	0	0	0	0	1	0	1	
<i>Morganella morganii</i> biogp 1	1	0	0	0	0	0	0	0	0	0	1	0	1	
<i>Photorhabdus asymbiotica</i>	1	0	1	0	1	1	0	0	1	1	0	0	1	
<i>Pragia fontium</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Serratia marcescens</i>	1	1	0	0	1	0	0	0	0	0	1	0	1	
<i>Proteus vulgaris</i>	3	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Photorhabdus luminescens</i> 25C	3	0	2	0	2	0	0	0	0	1	0	0	1	
<i>Proteus mirabilis</i>	10	2	2	1	5	1	0	1	2	1	1	1	3	
<i>Pantoea agglomerans</i> complex	13	2	5	3	10	4	0	2	6	0	2	1	3	
TOTAL	115	20	30	12	62	15	5	4	24	4	23	2	29	

<sup>a</sup>CDT/ DDT – isolates that are positive for both CDT and DDT tests; <sup>b</sup>MHT/ EDTA – isolates that are positive for both MHT and EDTA

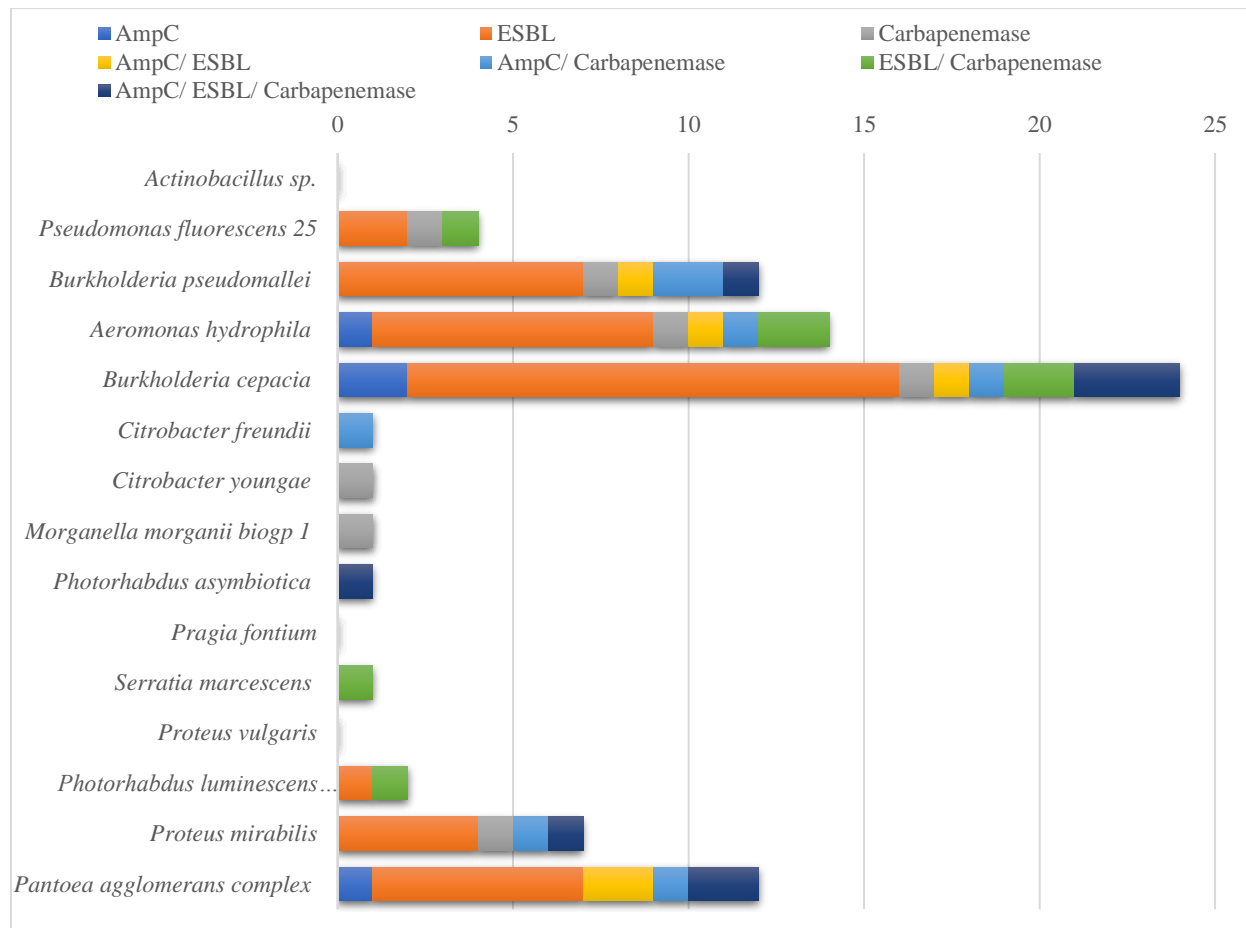


Fig 3: Co-production profile for selected Gram-negative bacteria isolates from HIV seropositive patients

#### Co-production of ESBL, AmpC, and carbapenemase:

The presence of either ESBL, AmpC, or carbapenemase or the co-production of two or all three enzymes was evaluated in a total of 80 isolates. Only 8 (10.0%) isolates produced all three enzymes and these include 3 *Burkholderia cepacia*, 2 *Pantoea agglomerans* complex, and 1 each of *Burkholderia pseudomallei*, *Photobacterium asymbiotica* and *Proteus mirabilis*. The co-production of two enzymes (either ESBL/AmpC, ESBL/carbapenemase, or carbapenemase/AmpC) was detected in 19 (23.8%) GNB isolates while the last 53 (66.2%) GNB isolates produced only 1 enzyme each (Table 4 and Fig 3).

#### Resistance pattern to beta-lactam antibiotics and production of ESBL, AmpC, and carbapenemase among GNB isolates

In all 12 isolates resistant to imipenem, 11 (10 of which were MDR) produced either ESBL, AmpC, carbapenemase, or co-produced two or all three enzymes. The last resistant isolate (*Burkholderia cepacia*) albeit MDR, did

not produce any of the enzymes screened for. Four of them produced 2 enzymes each (carbapenemase and AmpC, 2; AmpC and ESBL, 2) while 5 produced only one enzyme each (ESBL, 4; carbapenemase, 1). Only 2 of them (*Burkholderia cepacia*, 1; *Photobacterium asymbiotica*, 1) produced all the three enzymes.

Seventy-five of the enzyme producers were resistant to ampicillin, 8 of them produced all three enzymes (all MDR), 48 produced only one of the three (ESBL, 38; AmpC, 4; carbapenemase, 6) while the remaining 19 isolates produced two out of the three enzymes (carbapenemase and AmpC, 7; AmpC and ESBL, 4; and carbapenemase and ESBL, 8) (Table 3). Fifty-five of the isolates were resistant to piperacillin, 13 of them (all MDR) were negative for the three enzymes; 3 produced all the three enzymes; co-production of two enzymes was detected in 9 isolates (carbapenemase and AmpC, 4; AmpC and ESBL, 2; and carbapenemase and ESBL, 4) while the remaining 30 isolates produced only one enzyme each (ESBL, 23; AmpC, 2; carbapenemase, 5). Six out of

fifteen isolates resistant to piperacillin/tazobactam did not produce any of the enzymes, while none produced all three. Four co-produced two enzymes (carbapenemase and AmpC, 1; AmpC and ESBL, 1; carbapenemase and ESBL, 2) and 5 isolates produced one enzyme each (ESBL, 4; carbapenemase, 1).

## Discussion:

The rising prevalence of multiple resistance to previously effective antibiotics in GNB as a result of the production of enzymes that confer resistance to drugs has been a worrisome trend in the antibiotic treatment regimen for clinicians. This study was designed to phenotypically screen for the production of ESBLs, AmpC  $\beta$ -lactamase, and carbapenemase in selected GNB isolates cultured from HIV seropositive patients in southwestern Nigeria. We report a high level of resistance of the GNB to ampicillin (94.5%), a commonly prescribed beta-lactam antibiotic. This resistance is likely the result of beta-lactam hydrolysing enzymes as observed in this study. On the other hand, 10.9% of the GNB were resistant to imipenem. This rate is lower than that reported by Dumaru et al., (58) where imipenem resistance rates for different species ranged between 0% to 60%. Again, Jalalvand et al., (21) reported resistance rates of 90% and 96.4% to imipenem and meropenem respectively.

However, resistance rate to piperacillin/tazobactam and imipenem was 11.3% and 13.8% respectively for the enzyme-producing GNB isolates. Yet, imipenem was the most active of all the antibiotics tested against the selected bacterial isolates. This high activity is in line with a previous study carried out by Ogbolu et al., (44) where imipenem was also the most effective of all the drugs tested. This highlights the fact that despite the rising prevalence of resistance to imipenem reported globally (59-62), it is still probably a highly effective antimicrobial agent in the event of therapeutic failure of other commonly prescribed antimicrobials particularly in this environment where carbapenems have been reported as the established treatment choice for serious infections caused by ESBL producing GNB (63).

The proportion of MDR strains among the selected GNB isolates was high (93.6% of the isolates were MDR), and for the hydrolysing enzyme-producing strains, 91.3% were MDR. The high values of MAR indices for ESBL, AmpC, and carbapenemase-producing strains (98.8% with MAR  $\geq$  0.2) implies that these isolates were obtained from an antibiotic pressurised environment. This is most likely because the isolates were recovered from HIV-infected patients who

are immunocompromised and are probably in constant contact with the hospital environment.

A high rate of ESBL production (53.9%, 62/115) was detected among the isolates in the study. The presence of ESBL trait in MDR GNB, particularly from clinical samples may be significant because it could be an indication of colonisation, with high potential for transfer from one patient to another (65). Colonisation by ESBL producing GNB has been linked to prior exposure to antibiotics and hospitalisation (65) which predisposes the host to infections caused by these organisms (31).

The frequency of AmpC  $\beta$  lactamase and carbapenemase producers were not as high as that of ESBL, with only 24 (20.9%) and 29 (25.2%) respectively. However, the rate of ESBL and AmpC production in this study is much higher than previously reported rates in Nigeria; 9.3% ESBL rate in 2007 (66), 15.8% ESBL and 11.3% AmpC rates from northern Nigeria (7), and 15.8% ESBL and 9.6% AmpC rates in southern Nigeria (44). Our rates however agree with the report of Iroha et al., (42) and Yusuf et al., (46) both in Nigeria where the prevalence rate of ESBL production was 56.6% and 58.0% respectively. Very recently, Ugah and Udeani (67) reported ESBL production rate of 61.5% in Enterobacteriaceae isolates from south-eastern Nigeria. A study conducted in Iran (21) reported that 73.3% of carbapenem-resistant isolates were carbapenemase-producing ones. This high rate of production in our isolates could also be due to the immunocompromised nature of the patients. However, no ESBL production was detected in the two *Citrobacter* species, *Morganella morganii* biogp 1, and *Proteus vulgaris*; and no AmpC production was detected in *Citrobacter youngae*, *Morganella morganii* biogp 1, *Photorhabdus luminescens* 25C, *Pseudomonas fluorescens* 25 and *Serratia marcescens*.

Carbapenems have been reported to be the cornerstone of therapy for patients who have serious infections in which ESBL producers are implicated (63,68). The production of carbapenemases by some of the isolates although not remarkably high is quite worrisome because of the potential threat to the treatment regimen in the group of patients from which they were recovered. The production of ESBLs is a significant factor in the development of resistance of pathogenic GNB to broad-spectrum antibiotics, and the co-production of any of the three enzymes screened in this study portends serious implications especially for debilitated and immunosuppressed individuals such as HIV-infected patients. It also poses serious challenges for the treatment of opportunistic infections which is quite common in this group

of patients due to their immuno-compromised state.

In this study, there was co-production of two or more of the enzymes among the isolates. The highest number of strains that produced at least one of the enzymes was *Pantoea agglomerans*, formerly called, and identified by the Microbact 24E identification kit as *Enterobacter agglomerans*, 92.3% of the strains of this bacterium tested was a producer of at least one of the enzymes screened for and 2 of 13 strains tested produced all three enzymes. *Pantoea agglomerans* has been reported to be an opportunistic pathogen, especially in the immuno-suppressed persons such as neonates, premature infants, burns or multiply traumatised patients, and patients with leukaemia or those undergoing immunosuppressive therapy, and not excluding HIV-infected individuals. It has been known to cause a range of infections, including wound, blood stream, and urinary tract infections. Infections are typically acquired from infected vegetation penetrating the skin, or hospital-acquired when associated with the use of contaminated intravenous products due to its ability to grow in commercial infusion fluids (69) or exposure of hospitalised, often immuno-compromised individuals to medical equipment or fluids contaminated with this bacterium (70). Bloodstream infection can lead to disseminated disease, mainly septic arthritis, but also endophthalmitis, periostitis, endocarditis and osteomyelitis in humans (70).

Also worthy of note are the species, *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) and *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*) (71) in which 70.6% of each was capable of producing at least one of the enzymes (24/34 and 12/17 respectively). *B. cepacia* has been implicated in lung infections in patients with chronic granulomatous disease usually associated with pneumonia and septicaemia which are often associated with high fatality (72-75), although they rarely cause infection in the immunocompetent host. *B. cepacia* is known to be resistant to most antimicrobial agents and can acquire resistance against many more. As such, effective therapies are not clear-cut; decisions on the treatment of *B. cepacia* infections are made on a case-by-case basis, and prevention of infection is, therefore, the focus at management (76-78). *Burkholderia pseudomallei*, on the other hand, is a highly pathogenic organism that is known to cause an infection called melioidosis, a potentially life-threatening infectious disease affecting mammals, including humans (79). The organism is intrinsically resistant to a wide range of antimicrobials (80), and treatment with

ineffective antimicrobials may result in treatment failure leading to case fatality rates that may exceed 70% (81). Infections are often profoundly serious as clinical presentations may vary, and can culminate into diseases of the kidney, blood, heart, and other fatal disorders.

*Proteus mirabilis* is known to cause 90% of all *Proteus* infections in humans. It is thought that the majority of *P. mirabilis* urinary tract infections (UTI) are retrograde, resulting from the ascension of bacteria from the lower genitourinary tract, while others are by direct contact with infected persons, particularly in healthcare settings (82). *Proteus* species can also cause infection in the respiratory tract, eye, ear, nose, skin, throat, as well as in burns and wounds, mostly in hospitalised patients (82). *Photobacterium asymbiotica* has been reported to be infectious to humans, although infections are mostly non-fatal (83). It is pertinent to note that the most prevalent GNB isolates recovered in our study were opportunistic pathogens that generally occur as contaminants on environmental surfaces within the healthcare setting. This could probably be adduced to the fact that the population from which the isolates were recovered were immunosuppressed by HIV infection, and as such are prone to colonisation by these non-virulent organisms. Again, these patients pay regular visits to the hospital for clinic visitations, monitoring of their health and immune status, and for treatments when ill or experiencing symptoms associated with AIDS-related complexes.

The rapid detection of ESBLs in MDR organisms is highly essential to establish appropriate antibiotic treatment, as well as implement infection control measures. The KPCs, and OXA-48, are among the most prevalent carbapenemases worldwide (84) and have been reported to cause outbreaks. NDM-1 can spread widely, as the plasmids that carry the NDM-1 gene are of broad host range, implying that they can disseminate easily between other members of the Enterobacteriaceae and unrelated species (85). The horizontal transfer of the NDM-1 gene itself between different plasmids in the same organism has been documented (86).

A major limitation of our study was the inability to confirm the genes encoding ESBL, AmpC, and carbapenemase in our isolates with a molecular method which is the 'gold standard' for detecting the presence of genes responsible for the expression of these enzymes. This is particularly concerning as previous reports have shown that organisms sensitive to carbapenems in the phenotypic test sometimes harbour carbapenemase gene detected by polymerase chain reaction. Infections associated with such

wrongly characterized isolates have resulted in treatment failures due to limited antibiotic options, sporadic outbreaks of infections caused by these organisms, extended hospital stays and ultimately higher cost of treatment, as well as increased morbidity and mortality. The use of combination therapy with two active drugs, colistin/tigecycline with intravenous fosfomycin, has been recommended for the treatment of infection caused by these organisms (87), but more recently, fosfomycin, aminoglycosides, and temocillin were reported to be relatively effective, and may well substitute carbapenems for therapy in the treatment of ESBL producing Enterobacteriaceae (63).

In conclusion, this study reports high rate of ESBL, AmpC, and carbapenemase co-production in selected GNB pathogen. The EDTA method was more sensitive in detecting carbapenemase production while MHT was better at detecting AmpC enzyme. A high proportion of the enzyme producing GNB were MDR and had high MAR indices (98.8% with MAR  $\geq$  0.2), implying that the isolates had been exposed to a myriad of antibiotics. As such, routine checks for antibiotic hydrolysing enzyme production among bacterial isolates in clinical laboratories by phenotypic or genotypic methods should be embraced. Continuous surveillance, monitoring, screening, and reporting of isolates capable of producing these enzymes is essential to curtail the scourge of antibiotic resistance, as well as to understand better the mechanisms behind these traits.

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Copyright AJCEM 2021: <https://dx.doi.org/10.4314/ajcem.v22i1.7>**Original Article****Open Access****Prevalence and factors associated with extended-spectrum  $\beta$ -lactamase producing Enterobacteriaceae bacteraemia in University Hospital of Befelatanana, Madagascar**<sup>\*1</sup>Rakotovo-Ravahatra Z. D., <sup>2</sup>Randriatsarafara, F. M., <sup>3</sup>Rakotovo, A. L., and <sup>3</sup>Rasamindrakotroka, A.<sup>1</sup>Laboratory of Joseph Raseta Befelatanana, University Hospital, Antananarivo, Madagascar<sup>2</sup>Public Health Department, Faculty of Medicine, University of Antananarivo, Madagascar<sup>3</sup>Medical Biology Department, Faculty of Medicine, University of Antananarivo, Madagascar\*Correspondence to: [ravahatradomoina@yahoo.fr](mailto:ravahatradomoina@yahoo.fr); +261 34 09 301 20**Abstract:****Background:** The extended-spectrum  $\beta$ -lactamase (ESBL) producing Enterobacteriaceae are a major cause of nosocomial bacteraemia. The objectives of this study are to describe the antibiotic resistance pattern of ESBL producing Enterobacteriaceae responsible for bacteraemia and identify factors associated with these infections in a University Hospital in Madagascar.**Methodology:** This is a descriptive cross-sectional study of 300 randomly selected patients with clinical features of bacteraemia whose blood cultures were processed for isolation and identification of bacterial pathogens over a period of six months (October 2019 to March 2020) at the laboratory of the University Hospital of Befelatanana. Blood culture samples were processed by conventional microbiological method for isolation of Enterobacteriaceae, which were identified to species level using Analytical Profile Index (API) 20E® test system. Antibiotic susceptibility of each isolate was performed by the disk diffusion technique and ESBL production was detected by the 'synergy' method.**Results:** Of the 300 patients, 54 were positive for bacteria, giving a prevalence rate of 18% for microbiologically documented bacteraemia. Of the 54 bacterial pathogens, Enterobacteriaceae isolates constituted 37 (68.5%), with 23 (42.6%) being ESBL producing and 14 (25.9%) non-ESBL producing isolates, 14 (25.9%) were staphylococci and 3 (5.6%) were streptococci isolates. All 23 (100%) ESBL producing Enterobacteriaceae isolates were resistant to amoxicillin, amoxicillin-clavulanic acid and the third generation cephalosporins (3GC), 19 (82.6%) to gentamycin and 18 (78.3%) to cotrimoxazole. On the other hand, the non-ESBL producing isolates were more sensitive because only 10 (71%) were resistant to amoxicillin, 7 (50%) to cotrimoxazole, 2 (14%) to amoxicillin-clavulanic acid, 1 (7.1%) to gentamycin, and none (0%) was resistant to 3GC. All 54 Enterobacteriaceae isolates were sensitive to amikacin and imipenem. Age less than 20 years (93.8%) ( $p=0.001$ ) and hospitalization in intensive care units (90.9%) ( $p=0.04$ ) were significant risk factors associated with infection by ESBL producing Enterobacteriaceae.**Conclusion:** ESBL producing Enterobacteriaceae responsible for bacteraemia in University Hospital of Befelatanana, Madagascar, are resistant to many classes of antibiotics. Carbapenems and amikacin are the antibiotics of choice.**Keywords:** ESBL, Enterobacteriaceae, bacteraemia, antibiotic resistance

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**Prévalence et facteurs associés à la bactériémie à entérobactéries productrices de  $\beta$ -lactamases à spectre étendu dans l'hôpital universitaire de Befelatanana, Madagascar**<sup>\*1</sup>Rakotovo-Ravahatra Z. D., <sup>2</sup>Randriatsarafara, F. M., <sup>3</sup>Rakotovo, A. L., et <sup>3</sup>Rasamindrakotroka, A.<sup>1</sup>Laboratoire Joseph Raseta Befelatanana, Hôpital Universitaire, Antananarivo, Madagascar<sup>2</sup>Département de Santé Publique, Faculté de Médecine, Université d'Antananarivo, Madagascar

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## Abstrait:

**Contexte:** Les entérobactéries productrices de  $\beta$ -lactamases à spectre étendu (BLSE) sont une cause majeure de bactériémie nosocomiale. Les objectifs de cette étude sont de décrire le profil de résistance aux antibiotiques des entérobactéries productrices de BLSE responsables de bactériémie et d'identifier les facteurs associés à ces infections dans un hôpital universitaire de Madagascar.

**Méthodologie:** Il s'agit d'une étude transversale descriptive de 300 patients sélectionnés au hasard présentant des caractéristiques cliniques de bactériémie dont les hémocultures ont été traitées pour l'isolement et l'identification des bactéries pathogènes sur une période de six mois (octobre 2019 à mars 2020) au laboratoire du Hôpital universitaire de Befelatanana. Les échantillons d'hémoculture ont été traités par une méthode microbiologique conventionnelle pour l'isolement des entérobactéries, qui ont été identifiées au niveau de l'espèce à l'aide du système de test Analytical Profile Index (API) 20E®. La sensibilité aux antibiotiques de chaque isolat a été réalisée par la technique de diffusion sur disque et la production de BLSE a été détectée par la méthode «synergie».

**Résultats:** Sur les 300 patients, 54 étaient positifs pour les bactéries, ce qui donne un taux de prévalence de 18% pour une bactériémie microbiologiquement documentée. Parmi les 54 bactéries pathogènes, les isolats d'entérobactéries constituaient 37 (68,5%), 23 (42,6%) produisant des BLSE et 14 (25,9%) isolats ne produisant pas de BLSE, 14 (25,9%) étaient des staphylocoques et 3 (5,6%) l'étaient isolats de streptocoques. Les 23 isolats (100%) de BLSE produisant des Enterobacteriaceae étaient tous résistants à l'amoxicilline, à l'amoxicilline-acide clavulanique et aux céphalosporines de troisième génération (3GC), 19 (82,6%) à la gentamycine et 18 (78,3%) au cotrimoxazole. En revanche, les isolats non producteurs de BLSE étaient plus sensibles car seuls 10 (71%) étaient résistants à l'amoxicilline, 7 (50%) au cotrimoxazole, 2 (14%) à l'amoxicilline-acide clavulanique, 1 (7,1%) à la gentamycine, et aucun (0%) n'était résistants à la 3GC. Les 54 isolats d'Enterobacteriaceae étaient tous sensibles à l'amikacine et à l'imipénème. L'âge de moins de 20 ans (93,8%) ( $p=0,001$ ) et l'hospitalisation en unité de soins intensifs (90,9%) ( $p=0,04$ ) étaient des facteurs de risque importants associés à l'infection par les entérobactéries productrices de BLSE.

**Conclusion:** Les entérobactéries productrices de BLSE responsables de bactériémie à l'hôpital universitaire de Befelatanana, Madagascar, sont résistantes à de nombreuses classes d'antibiotiques. Les carbapénèmes et l'amikacine sont les antibiotiques de choix.

**Mots clés:** BLSE, entérobactéries, bactériémie, résistance aux antibiotiques

## Introduction:

Bacteraemia is defined as the presence of bacteria in the blood. The blood is normally sterile environment, therefore the detection of bacteria in the blood is always abnormal. The annual incidence of community-onset bacteraemia is reported to be between 40 and 154 per 100,000 populations (1). Bacteraemia is classified as nosocomial or community acquired, in accordance with the classic CDC criteria (2). Among bacterial isolates responsible for bacteraemia, the extended spectrum  $\beta$ -lactamase (ESBL) producing Enterobacteriaceae are highly implicated. Indeed, community and hospital-acquired ESBL producing Enterobacteriaceae are prevalent worldwide (3). Reliable identification of ESBL producing organisms in clinical laboratories can be challenging, therefore their prevalence is likely to be underestimated (3). ESBL are enzymes that confer resistance to most  $\beta$ -lactam antibiotics, including penicillins, cephalosporins, and monobactam (aztreonam). Infections caused by ESBL producing bacterial pathogens have been associated with poor clinical outcomes (3).

Bacteraemia have several important health consequences. The immune response to the bacteria can cause sepsis and septic shock especially in Gram negative bacteria, which is associated with high mortality (4).

Bacteria can also spread via the blood to other parts of the body, causing infections far away from the original site of infection, such as endocarditis and osteomyelitis (5). Antimicrobial therapy of bacteraemia caused by ESBL-producing organisms presents additional challenge, because these organisms are also often resistant to other antimicrobials such as trimethoprim-sulfamethoxazole, aminoglycosides and the fluoroquinolones.

Until recently, the major problems posed by ESBL-producing organisms were related to nosocomial infections caused by *Klebsiella pneumoniae*, which produced mainly the TEM and SHV types of ESBL (6). Similarly, ESBL production is one of the most important resistance mechanisms that hinder antimicrobial therapy of infections caused by the Enterobacteriaceae (7). Therefore, it is imperative to quantify the problem, and reinforce guidelines promoting appropriate antibiotic use. Antibiotic resistance studies of these ESBL-producing Enterobacteriaceae should hence be regularly conducted to improve patient management. The objectives of this study are to describe the antibiotic resistance profile of ESBL-producing Enterobacteriaceae responsible for bacteraemia and to identify risk factors associated with these infections in University Hospital of Befelatanana, Madagascar.

## Materials and method:

### Study setting

This study was carried out at the laboratory of the University Hospital of Befelatanana, located in Antananarivo, the capital city of Madagascar. This laboratory is versatile and performs haematological, immunological, biochemical and microbiological analyses on clinical samples.

### Study design, subjects and sample size

This study was a descriptive cross-sectional design involving 300 patients with clinical suspicion of blood stream infections (BSI) randomly recruited for the study over a period of six months (October 2019 to March 2020), and whose blood culture samples were processed in the laboratory of the University Hospital of Befelatanana. BSI was confirmed in subjects who had clinical features compatible with systemic inflammatory response syndrome, and isolation of bacteria in at least one blood culture bottle (8).

### Blood specimen collection

Blood samples were collected from the antecubital fossa of each patient at the peak of fever by a trained nurse under strict aseptic condition using sterile needles and syringes. Three blood samples per patient (paired samples of aerobic and anaerobic) were collected within 24 hours, and between 5 to 10 ml of blood was collected for each sample. The samples were inoculated into the blood culture bottles (Oxoid B0100M®, UK) using new set of sterile needles with thorough mixing of the content, for aerobic, anaerobic and micro-aerophilic incubation. The inoculated bottles were transported immediately to the laboratory.

### Processing of blood culture for isolation of Enterobacteriaceae

Blood culture was performed with the conventional culture technique. All inoculated culture bottles were incubated at 37°C in aerobic, anaerobic and micro-aerophilic environment. Culture bottles were examined daily by laboratory technicians for evidence of bacterial growth, which is shown by positive pressure growth indicator device connected to the culture bottles. A positive pressure in the bottle displaces a quantity of blood/broth mixture into the chamber as a sign of microbial activity, and this was indicated when the blood/broth mixture rises above the green locking sleeve of the growth indicator device. Blood culture result was adjudged negative if there was no evidence of growth after 5 days of incubation

### Bacteria identification from positive blood cultures

Positive blood cultures were sub-cultured on blood, chocolate and chromogenic agar (Uriselect®) to obtain discrete colonies. The Uriselect® agar was used to preliminarily identify Enterobacteriaceae through specific staining of bacterial colonies on this medium. The confirmatory biochemical identification of the Enterobacteriaceae was done using the Analytical Profile Index (API) 20E® test system.

### Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was performed on Mueller-Hinton agar using the disk diffusion method in accordance with the recommendations of the "Comité de l'antibiogramme de la Société Française de Microbiologie" (9). The MH agar plate was inoculated with 0.5 McFarland standards suspension of each Enterobacteriaceae isolate, and antibiotic disks were placed on the plate and incubated aerobically at 37°C for 24 hours. The diameter of zone of inhibition to each disk was read using a calibrated ruler and the result interpreted as sensitive or resistant according to the guidelines of the "Comité de l'antibiogramme de la Société Française de Microbiologie" (9).

The antibiotic disks used included amoxicillin (20µg), amoxicillin-clavulanic acid (20µg/10µg), 3rd generation cephalosporins (cefixime 5µg, ceftazidime 30µg, ceftriaxone 30µg, and cefotaxime 30µg), imipenem (10µg), aminoglycosides (gentamycin 10µg, tobramycin 10µg, and amikacin 30µg), quinolones (nalidixic acid 30µg, ciprofloxacin 5µg, and levofloxacin 5µg), trimethoprim-sulfamethoxazole (1.25µg/23.75µg) and chloramphenicol (30µg).

### Detection of ESBL production

ESBL production was detected by the 'synergy' method using amoxicillin/clavulanic acid disk (20/10µg) placed at 30 mm center to center of ceftazidime disk (30µg), cefotaxime disk (30µg), ceftriaxone disk (30µg) and aztreonam disk (30µg) on MH agar plate that has been inoculated with 0.5 McFarland standards suspension of the Enterobacteriaceae isolates. After 24 hours aerobic incubation at 37°C, a "champagne cork" image indicates ESBL production (10). *Klebsiella pneumoniae* ATCC 700603 was used as control strain (11).

### Data collection, entry and statistical analysis

The study parameters of each subject obtained were gender, age, unit/department, bacteriological results of blood culture and the results of antibiogram, which were obtained

from the laboratory case files. Each patient had previously completed analysis request form which contains all the required demographic and clinical information, which were transcribed into the laboratory case files. Data entry and processing were performed on EPI-INFO 3.5.2 statistical software. The comparison of variables was done using the Chi square test, and statistical significance threshold used was  $p < 0.05$ .

#### Ethical approval

The study was approved by the Director of Establishment of the University Hospital of Befelatanana and the Department Head of the Laboratory. This study respected the notion of anonymity and confidentiality.

#### Results :

Of the total of 300 patients with clinical evidence of blood stream infections

recruited in this study, blood culture sample was positive for bacteria in 54 patients, giving a prevalence rate of 18% for bacteraemia. Of the 54 bacterial isolates, 37 (68.5%) were Enterobacteriaceae with 23 (42.6%) ESBL producing and 14 (25.9%) non-ESBL producing, 14 (25.9%) were staphylococci and 3 (5.6%) were streptococci isolates (Fig 1).

The 23 ESBL producing Enterobacteriaceae were represented by 11 (47.8%) *Klebsiella pneumoniae*, 8 (34.8%) *Enterobacter cloacae* and 4 (17.4%) *Escherichia coli* isolates (Fig 2). The 14 non-ESBL producing isolates were represented by 6 (42%) *E. coli*, 3 (21.4%) *E. cloacae*, 2 (14.3%) *K. pneumoniae*, 1 (7.1%) *Serratia marcescens*, 1 (7.1%) *Salmonella Typhi* and 1 (7.1%) *Shigella dysenteriae* isolates (Fig 3).

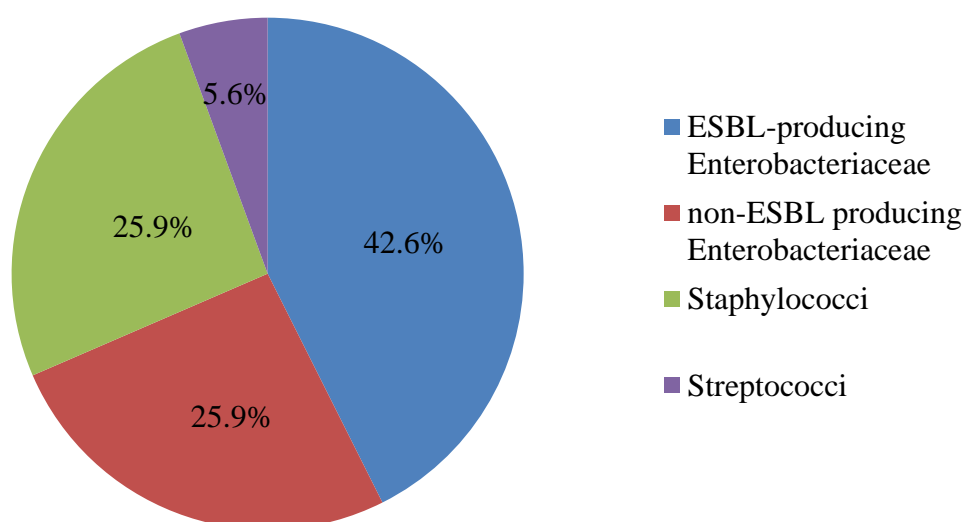


Fig 1: Bacteriological results of the blood culture among patients with bacteraemia in University Hospital, Befelatanana, Madagascar

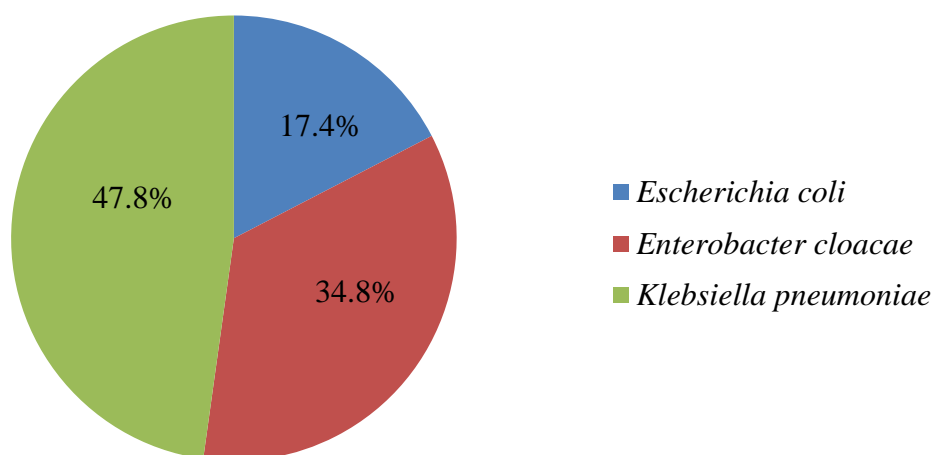


Fig 2: Species of ESBL producing Enterobacteriaceae causing bacteraemia in University Hospital, Befelatanana, Madagascar

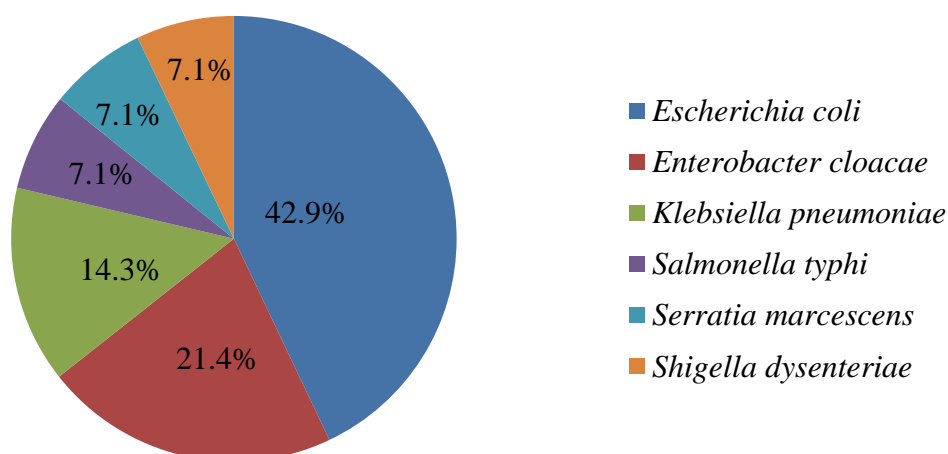


Fig 3: Species of non-ESBL producing Enterobacteriaceae isolates causing bacteraemia in University Hospital, Befelatanana, Madagascar

Regarding antibiotic resistance of the Enterobacteriaceae, resistance rate varies from 0% to 100% for all of the antibiotics tested. All 23 (100%) ESBL producing Enterobacteriaceae were resistant to amoxicillin, amoxicillin-clavulanic acid, and third generation cephalosporins, 19 (82.6%) were resistant to gentamycin and 18 (78.3%) to cotrimoxazole. On the other hand, the non-ESBL producing Enterobacteriaceae were more sensitive because only 10 (71%) were resistant to amoxicillin, 7 (50%) to cotrimoxazole, 2

(14%) to amoxicillin-clavulanic acid, 1 (7.1%) to gentamycin and no isolate was resistant to third generation cephalosporins.

All Enterobacteriaceae isolates were sensitive to amikacin and imipenem (Fig 4). Age below 20 years (15 of 16 patients, 93.8%) ( $p=0.001$ ), and hospitalization in intensive care units (10 of 11 patients, 90.9%) ( $p=0.04$ ) were significantly associated with ESBL producing Enterobacteriaceae bacteraemia (Table 1).

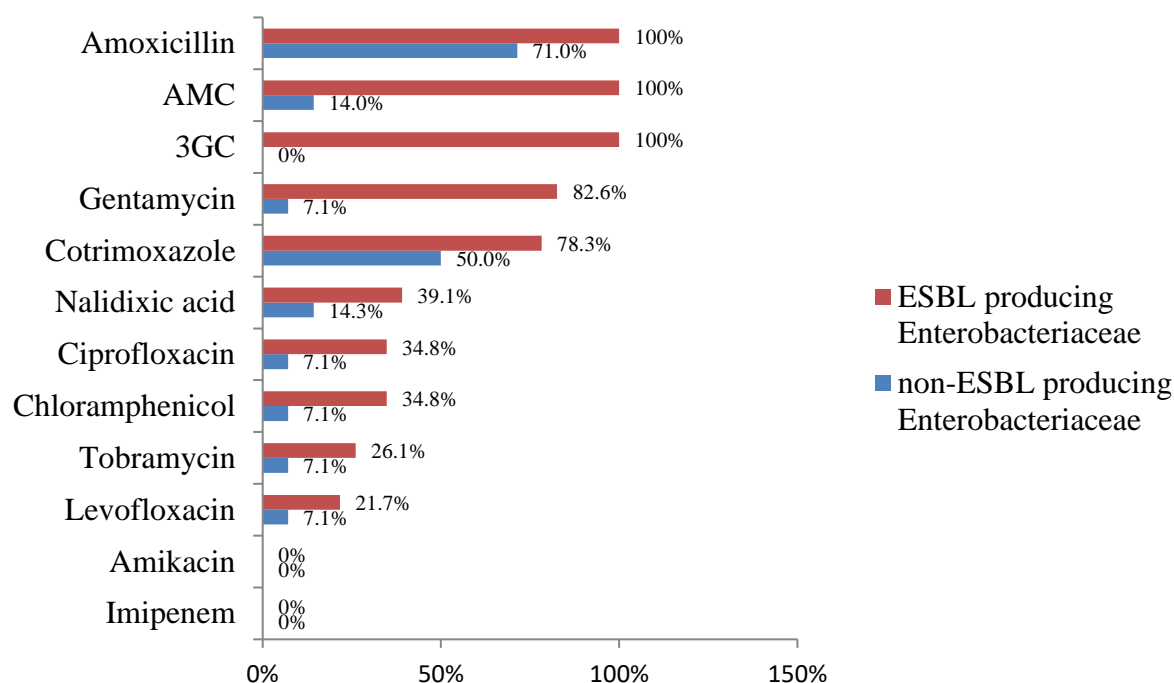


Fig 4: Antibiotic resistance of ESBL and non-ESBL producing Enterobacteriaceae causing bacteraemia in University Hospital, Befelatanana, Madagascar

Table 1: Factors associated with ESBL-producing Enterobacteriaceae bacteraemia in University Hospital, Befelatanana, Madagascar

	ESBL-producing Enterobacteriaceae (n=23)		Non-ESBL producing Enterobacteriaceae (n=14)		Total (n=37)	p value
	N	%	N	%		
Age (years)						
>20	8	38.1	13	61.9	21	0.001 <sup>s</sup>
≤ 20	15	93.8	1	6.3	16	
Gender						
Female	15	62.5	9	37.5	24	0.76 <sup>ns</sup>
Male	8	61.5	5	38.5	13	
Department						
Intensive care unit	10	90.9	1	9.1	11	0.04 <sup>s</sup>
Internal medicine	13	50.0	13	50.0	26	

s=statistically significant; ns=not statistically significant

## Discussion:

This study reports a prevalence rate of 18% for microbiologically documented bacteraemia in University Hospital of Befelatanana, Madagascar, which included patients of all age groups in different departments/units of the hospital. The rate in our

study is higher than the 3.3% reported by Jamil et al., (12) among patients with chronic kidney disease, and 2.9% reported by Biondi et al., (13) among febrile neonates. However, the prevalence rate in our study is lower compared to the study conducted by Adeyemo et al., (14) who reported bacteraemia prevalence rates of 40.9%, 33.3%, and



50% in cleft lip surgery, cleft palate surgery, and alveoloplasty respectively. Indeed, bacteria can enter the blood stream as a severe complication of infections such as pneumonia or meningitis, during surgery (especially involving mucous membranes such as gastrointestinal tract), or due to catheters and other foreign bodies inserted into the blood vessels, including during intravenous injection drug abuse (15). Transient bacteraemia can also result after dental procedures or brushing of teeth (16).

Our study reported 42.6% (23 of 54) Enterobacteriaceae isolates causing bacteraemia to be ESBL-producing. This is lower than 73.3% rate reported among 131 bacteraemic patients in a study carried out in South India (7). Indeed, we found many other bacteria responsible for bacteraemia in this study such as streptococci and staphylococci. ESBLs are plasmid encoded  $\beta$ -lactamases that confer resistance to penicillins, cephalosporins, and aztreonam, which are frequently found in *Klebsiella* species, *E. coli*, and other enterobacteria (6). Our study reported high frequency of ESBL-producing Enterobacteriaceae among species of *K. pneumoniae*, *E. coli* and *E. cloacae* but some species were non-ESBL producing isolates and these included *S. marcescens*, *S. Typhi* and *S. dysenteriae*.

The antibiotic susceptibility (AST) profile indicated that the ESBL producing Enterobacteriaceae isolates were more resistant than the non-ESBL producing isolates. In addition to their resistance to majority of the  $\beta$ -lactam agents, the ESBL producing isolates were also more resistant to other classes of antibiotics such as cotrimoxazole, aminoglycosides (gentamycin, tobramycin), quinolones (nalidixic acid, ciprofloxacin, levofloxacin) and chloramphenicol, than the non-ESBL producing isolates. Indeed, ESBL producing Enterobacteriaceae are frequently found in hospital and are responsible for several nosocomial infections (17). These antibiotic resistant bacteria had emerged under the pressure of frequent use and misuse of certain antibiotics in the hospitals. Cefotaxime is one of the most commonly used oxyimino- $\beta$ -lactam, a fact that may have favored the selection of CTX-M ESBL producing isolates, because such types of ESBL are mainly cefotaximases (6,18).

The fluoroquinolone use and misuse, which is also frequent, has been identified as a risk factor for other infections caused by ESBL-producing *E. coli* (19,20), probably as a result of co-selection (21). The empiric use of the 3rd and 4th generation cephalosporins should be curtailed, as cephalosporin use was associated with an increased risk of ESBL production. A study conducted by Hsieh et al., (22) showed that significant risk factors associated with bacteraemia by ESBL produ-

cing *E. coli* included recent antibiotic exposure (within 30 days) and urinary catheter placement. In view of their excellent *in vitro* activity, carbapenems should be the initial empiric choice for serious life-threatening infections caused by Enterobacteriaceae, with prompt de-escalation when culture and susceptibility results become available (7). Indeed, no Enterobacteriaceae isolate was resistant to carbapenems (imipenem) in our study, and similarly, all isolates were susceptible to amikacin. A study in Douala also found that imipenem (with 1.3% resistance) and amikacin (with 12.9% resistance rate) were the most effective antibiotics against enterobacteria (23). Taking together, these data suggest that the penems (carbapenem, imipenem, meropenem) and aminoglycosides especially amikacin could be an effective alternative choice for the treatment of ESBL-producing Enterobacteriaceae bacteraemia.

With regards to risk factors for Enterobacteriaceae bacteraemia, subjects under 20 years of age and those hospitalized in the intensive care units were most frequently infected by ESBL-producing isolates ( $p < 0.05$ ). Studies have shown that children are more fragile and more likely to be infected by ESBL producing Enterobacteriaceae (24). Similarly, invasive procedures such as mechanical ventilation, tracheostomy or the use of catheters favor nosocomial infections including bacteraemia in the intensive care units (25). Thus, infection prevention and control measures must be reinforced in intensive care units to limit the spread of these resistant bacteria.

## Conclusion:

ESBL producing Enterobacteriaceae are frequently responsible for nosocomial bacteraemia in Madagascar. These bacteria are resistant to many classes of antibiotics, while carbapenems and amikacin appear effective, and are the antibiotics of choice. Infection prevention and control measures must be reinforced in intensive care units to limit further emergence and spread of resistant bacteria.

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## Original Article

## Open Access

## Antimicrobial Stewardship Implementation in Nigerian Hospitals: Gaps and Challenges

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### Abstract:

**Background:** Antimicrobial resistance (AMR) is a major clinical challenge globally. It is mainly a consequence of inappropriate prescribing and use of antibiotics. Antimicrobial stewardship (AMS) ensures that antibiotics are prescribed and used appropriately. This study assessed AMS practice in selected Nigerian hospitals.

**Methodology:** This was a cross sectional survey of 20 Federal, State and Private tertiary hospitals randomly selected from the six geopolitical zones of Nigeria. Using an adapted WHO tool on AMS, data were collected from each hospital as regard the existence of AMS committee, Accountability and Responsibility, AMS actions, Education and Training, Monitoring and Evaluation, Infection Prevention and Control (IPC) practice, facilities to support AMS, and challenges to AMS implementation. Gaps and challenges to the implementation of the AMS among the hospitals were identified.

**Results:** Only 6 (30%) of the 20 hospitals had AMS committees while 2 (10%) had any evidence of leadership commitment to AMS. All the hospitals had laboratory facilities to support culture and sensitivity testing. There were no regular AMS-related education or training, monitoring, evaluation or reporting activities in the hospitals, except in 7 (25%) that had participated in the global point prevalence survey (Global-PPS) of antimicrobial use and resistance being hosted by the University of Antwerp, Belgium. Challenges impeding AMS activities included lack of human and financial resources, prescribers' opposition, lack of awareness and absence of AMS committees. Most of the gaps and challenges bordered on seeming lack of knowledge and inadequate communication among prescribers and other stakeholders.

**Conclusion:** There is need for intense education and training activities for prescribers and other stakeholders, including but not limited to hospital administrators.

**Keywords:** Survey, Antimicrobial Stewardship, Antimicrobial Resistance; Nigeria

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## Mise en œuvre de la gestion des antimicrobiens dans les hôpitaux Nigériens: lacunes et défis

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## Abstrait:

**Contexte:** La résistance aux antimicrobiens (RAM) est un défi clinique majeur à l'échelle mondiale. C'est principalement une conséquence d'une prescription et d'une utilisation inappropriées d'antibiotiques. La gestion des antimicrobiens (AMS) garantit que les antibiotiques sont prescrits et utilisés de manière appropriée. Cette étude a évalué la pratique de l'AMS dans certains hôpitaux Nigériens.

**Méthodologie:** Il s'agissait d'une enquête transversale de 20 hôpitaux tertiaires fédéraux, d'État et privés sélectionnés au hasard dans les six zones géopolitiques du Nigéria. À l'aide d'un outil OMS adapté sur l'AMS, des données ont été collectées auprès de chaque hôpital en ce qui concerne l'existence d'un comité AMS, la responsabilité et la responsabilité, les actions AMS, l'éducation et la formation, le suivi et l'évaluation, la pratique de prévention et de contrôle des infections (IPC), les installations pour soutenir l'AMS, et les défis de la mise en œuvre de l'AMS. Les lacunes et les défis liés à la mise en œuvre de l'AMS parmi les hôpitaux ont été identifiés.

**Résultats:** Seuls 6 (30%) des 20 hôpitaux avaient des comités AMS tandis que 2 (10%) avaient des preuves d'engagement du leadership envers l'AMS. Tous les hôpitaux disposaient d'installations de laboratoire pour soutenir la culture et les tests de sensibilité. Il n'y avait pas d'activités régulières d'éducation ou de formation, de suivi, d'évaluation ou de rapportage liées à la MGS dans les hôpitaux, sauf dans 7 (25%) qui avaient participé à l'enquête mondiale sur la prévalence ponctuelle (Global-PPS) de l'utilisation et de la résistance aux antimicrobiens organisée par l'Université d'Anvers, Belgique. Les défis entravant les activités de l'AMS comprenaient le manque de ressources humaines et financières, l'opposition des prescripteurs, le manque de sensibilisation et l'absence de comités AMS. La plupart des lacunes et des défis se limitaient à un manque apparent de connaissances et à une communication inadéquate entre les prescripteurs et les autres intervenants.

**Conclusion:** Des activités d'éducation et de formation intensives sont nécessaires pour les prescripteurs et autres intervenants, y compris, mais sans s'y limiter, les administrateurs d'hôpitaux.

**Mots clés:** enquête, gestion des antimicrobiens, résistance aux antimicrobiens; Nigeria

## Introduction:

Antimicrobial resistance (AMR) has become a major challenge in clinical practice especially in the management of bacterial infections. The discovery of penicillin by Fleming in 1928 revolutionized medicine and brought hope of eradicating infections as exemplified by the infamous quote allegedly made by the United States Surgeon General Dr. William H. Stewart about 1967-1969, for which no source has been traced till date (1,2). Notwithstanding, the quote "*it is time to close the books on infection and declare the war against pestilence won*" was often used to underscore the ever-increasing challenge of antibiotic-resistant and emerging infections.

The tendency of bacteria to develop resistance to antibiotics and its likely negative impact was revealed by Fleming in his Nobel prize award lecture in 1945 (3). Presently many

common clinical isolates have developed one form of resistance or the other to most of the commonly used antibiotics and this has led to increased morbidity, mortality, length of hospital stay and healthcare cost. Between 1996 and 2018, the prevalence of methicillin resistance in *Staphylococcus aureus* rose from below 10% to above 50% in many countries in Africa, including Nigeria (5-8). It is estimated that at present, antibiotic resistance kills about 700,000 yearly worldwide, and that this figure has been projected to rise to about 10 million by 2050, with attendant economic crises (4).

Several studies have identified antibiotic selective pressure as the single most important driver of resistance in bacteria. The selective pressure results from use of antibiotics, particularly when used inappropriately (9,10). In a study by Hecker et al., (11), it was reported that 576 (30%) of a total of 1941 antimicrobial days of therapy prescribed for 129 patients were

unnecessary. The most common reasons for the unnecessary therapy included longer therapy than recommended (33%), non-infectious/non-bacterial syndrome (32%), colonizing or contaminating organisms (16%) and redundant prescriptions (10%). Similarly, antibiotic use has been found to correlate with antibiotic resistance (12-15); the more antibiotics are used, the more resistance develops and vice-versa. The World Health Organisation (WHO) estimates that by 2050, antimicrobial resistance will cost the world about \$100 trillion per annum if left unchecked (16). The WHO has also warned that the world is running out of antibiotics, and therefore urgent action is required for remedy (17).

To stem the emergence and rise in AMR and reserve drugs for the future, rational antibiotic use in humans and animal husbandry was recognized as a key strategy (18,19). The antimicrobial stewardship (AMS) strategy was introduced to ensure that only the right patient is given the right antibiotics for the right indication in the right dose for the right duration using the right route with the goals of reducing emergence of resistance, morbidity, mortality, length of hospital stay and healthcare cost due to infectious diseases (19,20). This strategy is in use in many parts of the world, especially in the developed countries. In Nigeria, the availability of antimicrobial stewardship programmes (ASPs) in hospitals is grossly inadequate and antimicrobial prescribing patterns are largely empirical (21).

This cross-sectional survey of Federal, State and Private tertiary hospitals in Nigeria was therefore designed to identify gaps and challenges that have stood against AMS practice in the country, the result of which is expected to serve as guide for stakeholders in planning for and implementing AMS programmes to improve the quality of healthcare and safety of patients.

## Materials and method:

### Study setting

The study setting is the Nigeria health system, which is structured in a tiered hierarchical manner; primary, secondary, and tertiary hospitals. These hospitals are under the controls of different administrative levels and provide varying complexity of services. The tertiary hospitals are the most specialized and are often under the control of the Federal (mostly) and some State Government, as well as few Private (Faith-based) organizations. They are often the most funded and have specialized manpower to provide tertiary level health care, training and research. They comprise the university teaching

hospitals, federal medical centers, and specialized hospitals such as the neuropsychiatric and the orthopaedic hospitals. These hospitals often have well equipped laboratory services supporting their service provision and research.

### Study design and sampling technique

This was a cross sectional survey of 20 out of 40 Federal, State and Private tertiary hospitals in Nigeria offering general acute and long-term cares and excluding the specialized hospitals. The list of all the Federal, State and Private tertiary hospitals was generated, and 3 hospitals were selected by balloting from each of the six geopolitical zones of the country. One additional hospital each was selected from two geopolitical zones with the highest number of tertiary hospitals. Only tertiary hospitals were included as they stood any chance of carrying out bacterial cultures, antimicrobial susceptibility and AMS given the resources available to them. The selected hospitals were then invited to participate in the survey

### Data collection instrument and analysis

A semi-structured questionnaire adapted from the WHO template (22) was e-mailed by the principal investigator from the central study site to each selected centre through an identified focal person who completed the questionnaire for the participating centre and returned for central collation and analysis. The questionnaire had different sections which assessed the existence of AMS committee in the hospital, Accountability and Responsibility, AMS actions, Education and Training, Monitoring and Evaluation, Infection Prevention and Control (IPC) practice, facilities to support AMS, and challenges to AMS implementation.

Gaps and challenges facing the different hospitals in implementing AMS were identified. The responses were summarized and presented in frequency tables using Microsoft Excel 2016.

## Results:

Of the 20 hospitals that participated in the survey, AMS committees were in existence in only 6 (30%) hospitals. Two hospitals (10%) had written evidence of leadership commitment while resource allocation by management and AMS policy document existed in only 1 (5%) hospital (Table 1).

All the AMS committees in the 6 hospitals comprise multidisciplinary members with clearly defined leadership. Terms of reference were available in only 2 of the 6 (33.3%) hospitals, while none has been having regular meetings of the committee or providing regular reports. Whereas 5 (25%) of the 20 hospitals

had IPC committees, only 1 (5%) had an AMS-IPC interface (Table 1).

Only 1 (5%) hospital had any form of treatment guidelines in place. None had antibiotic approval or restriction policy or audit, and none gave regular feedback to prescribers. Hospital formulary was available in 4 (20%) hospitals. One hospital has had training for prescribers and other stakeholders as at the time of the survey, but it was not sustained or regular. The only form of monitoring and evaluation in place was the periodic participation in the global point prevalence survey (Global-PPS) of antimicrobial consumption and resistance hosted by the University of Antwerp,

Belgium, by a total of 7 (35%) hospitals. Prescription sheets, drug charts and laboratory facilities for culture and antibiotic susceptibility were available in all the hospitals, while information technology (IT) facilities were available in 11 (55%). None of the charts or prescription sheets were standardized for AMS activity. Antibiotic policy was present in 2 (10%) hospitals (Table 1).

All hospitals identified some challenges to AMS implementation, which included lack of awareness, non-commitment of management, prescribers' challenge, and lack of human and financial resources (Table 2).

Table 1: Availability of AMS practices and identified gaps from hospitals in Nigeria

AMS Elements	Components of the AMS Elements	Present/Available n = 20	
		Yes (%)	No (%)
Antimicrobial Stewardship Committee	Existence of AMS Committees	6 (30)	14 (70)
	Written evidence of leadership commitment	3 (15)	17 (85)
	Resource Allocation	1 (5)	19 (95)
	AMS identified as priority	1 (5)	19 (95)
	AMS policy document	1 (5)	19 (95)
Accountability and Responsibility	Multidisciplinary AMS Committee	6 (30)	14 (70)
	AMS Terms of Reference	2 (10)	18 (90)
	Regular Meetings	0	20 (100)
AMS Actions	Treatment Guidelines	1 (5)	19 (95)
	Antibiotic Approvals/Restrictions	0	20 (100)
	Antibiotic Audit	0	20 (100)
	Hospital Formulary	4 (20)	16 (80)
Education and Training	Training for Prescribers and other AMS stakeholders	1 (5)	19 (95)
Monitoring and Evaluation	Indication, Dose, Duration, Route Monitoring	0	20 (100)
	Surveillance	7 (35)	13 (65)
Reporting and Feedback	Regular reports to prescribers and others, and Feedbacks	0	20 (100)
AMS Support Facilities	Clinical laboratories for culture and sensitivity	20 (100)	0
	Prescription sheets	20 (100)	0
	Drug charts	20 (100)	0
	Standardized drug chart and prescription sheet	0	20 (100)
	IT Facilities	11 (55)	9 (45)
IPC Activity	Antibiotic policy	2 (10)	18 (90)
	IPC Committee	5 (25)	15 (75)
	AMS-IPC Interaction	1 (5)	19 (95)

Table 2: Identified challenges impeding AMS practice in Nigerian hospitals

Challenges	No of Hospitals (%)
Lack of Funding	15 (75)
Poor awareness of AMS usefulness by staff	12 (60)
Prescribers' opposition	11 (55)
Lack of IT Facilities	9 (45)
No ASP committee	9 (45)
Higher priorities	8 (40)
Lack of Staff	8 (40)
Lack of Leadership Support	7 (35)
Administration not aware of programme	6 (30)

ASP =Antimicrobial Stewardship Programme; IT= Information Technology

## Discussion:

This cross-sectional survey was designed to identify factors hindering the practice of AMS in Nigeria tertiary hospitals. The results of our survey showed poor state of AMS practice among tertiary hospitals in Nigeria. Both the core and supplementary strategies were either non-existent or poorly practiced. The poor state of AMS practice is evidenced by the few AMS committees and near-total lack of leadership support and resource allocation. The health system has not identified AMS as a priority, hence the absence of policy and lack of resource allocation. This level of practice could explain the high level of antimicrobial use earlier reported in some Nigerian hospitals (23-26).

The few hospitals with AMS committees do not have any terms of reference and do not hold regular meetings. This could also partly explain the absence of leadership commitment or the reason for it. It will be challenging to operate a successful and sustainable AMS programme without leadership support and a functional AMS committee to drive the process. The near absence of AMS actions or interventions such as education and training, monitoring and evaluation, reporting and feedback might all be due to the absence of functional AMS committees, and leadership/administration support.

Although the six hospitals with AMS committees had multidisciplinary members with clear leadership, this has not engendered the implementation of the supplementary strategies such as education and training, which calls to question the level of knowledge about AMS of the membership of these committees. An interesting aspect of the situation in these hospitals

is the availability of adequate facilities for culture and sensitivity. Paradoxically, a previous study in Nigeria had identified poor use of the clinical microbiology laboratory by physicians (27). Some of the reasons advanced for the finding in this previous study included the belief by many physicians that "clinical diagnosis was sufficient", frustration at the "delay in getting results", physician having sufficient "knowledge of potent antibiotics", lack of access to clinical laboratory facilities, and non-availability of pathologists to assure the quality of laboratory tests. Under this scenario, a substantial number of prescriptions are bound to be or remain empiric and inappropriate, a situation that fuels antimicrobial resistance. In the absence of any significant AMS activity, there was nothing to monitor or evaluate. The participation in the global point prevalent survey was only a starting point for monitoring and evaluation activity.

The close relationship between AMS and IPC is well established (22,28,29). Despite the availability of IPC committee in five hospitals, AMS-IPC interaction holds only in one hospital, suggesting lack of appreciation of the need for this. The absence of AMS committee in some of the hospitals could also have accounted for the absence of this valuable handshake. Alongside the huge AMS deficits highlighted here, there are also some notable challenges to implementation of AMS in the hospitals. These include but not limited to lack of funding, poor awareness of AMS usefulness by staff, prescribers' opposition, and lack of leadership support. Similar findings have been reported in a previous study of eleven hospitals in six continents (30,31), where it was shown that, amongst others, the administrations in some of the study sites were not aware



of the programme. It would therefore be difficult for the leadership to support a programme it was never aware of. This emphasizes the need for adequate and proper communication to ensure that all relevant interest groups and stakeholders are involved for a functional and sustainable AMS programme.

## Conclusion:

In conclusion, AMS, a proven strategy in curbing AMR, is established in only few tertiary health facilities in Nigeria. This survey identified gaps and factors impeding the implementation of this strategy to include lack of education, training, and proper communication. If these gaps are addressed, it will aid the implementation and sustenance of AMS in these hospitals through appropriate measures that deal with issues of leadership support, lack of funding, prescribers' opposition, and low awareness of AMS usefulness by staff. The challenges of poor knowledge, implementation strategies, monitoring, reporting and feedback will also be largely addressed.

## Conflicts of interest:

Authors declare no conflicts of interest

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

## Long term outcomes of highly active antiretroviral therapy in HIV infected Nigerians and those co-infected with hepatitis B and C viruses

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**Abstract:**

**Background:** HIV co-infection with hepatitis B (HBV) and/or hepatitis C virus (HCV) is common, largely due to shared routes of transmission, but paucity of data exists for long term treatment outcomes of HIV infected patients, and those co-infected with HBV and HCV despite the high burden in Nigeria. The aim of study was to describe the long-term treatment outcomes in HIV infected Nigerians and to assess the effect of HBV and HCV co-infections on long-term response to antiretroviral therapy (ART).

**Methodology:** This was a retrospective study of HIV infected adults (> 18 years old) consecutively initiating ART between July 2004 and December 2007, who were followed up for 7 years (2011 and 2014). HBV and HCV infections were diagnosed by detection of serum hepatitis B surface antigen (HBsAg) and HCV antibody (HCVAb) respectively. HIV viral load and CD4 count were monitored 3-monthly after initiating ART, and treatment outcomes based on these were compared between patients with HIV mono-infection, HIV/HBV, HIV/HCV and HIV/HBV/HCV co-infections. Clinical and laboratory data of the patients were abstracted from the medical databases, FileMaker Pro, v 10, entered into Microsoft Excel, and analyzed using SPSS version 20.0.

**Results:** A total of 2,800 adults were evaluated (median age of 35.5 years; 64.2% female), of whom 197 (7.0%) were co-infected with HBV, 53 (1.9%) with HCV, and 15 (0.5%) with HBV and HCV. During the 7-year period, 369 (13.2%) patients were lost to follow up. Immune reconstitution, measured by CD4 recovery, was lower in both HBV and HCV co-infections compared to HIV mono-infection, but this was not statistically significant ( $p > 0.05$ ). Median baseline HIV viral load was 4.63 log copies/ml for all groups, which decreased to undetectable level at a median time of 6 months and remained so for the study duration.

**Conclusion:** This study revealed a higher virologic failure among HIV/HCV co-infected group compared to other groups. No immunological difference in ART treatment outcomes between HIV mono-infected and those co-infected with HBV and HCV after 7-year follow-up. Gradual rise in CD4 was found to be an immunological evidence of the body's recovery from HIV, buttressed by the drop in viral load over the 7-year period.

**Keywords:** ART, HIV, HBV, HCV co-infection, long term outcomes

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## Résultats à long terme du traitement antirétroviral hautement actif chez les Nigériens infectés par le VIH et ceux co-infectés par les virus des hépatites B et C

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## Abstrait:

**Contexte:** La co-infection par le VIH avec l'hépatite B (VHB) et/ou le virus de l'hépatite C (VHC) est courante, en grande partie en raison des voies de transmission partagées, mais il existe peu de données sur les résultats du traitement à long terme des patients infectés par le VIH, et ceux co -infectés par le VHB et le VHC malgré le fardeau élevé au Nigéria. Le but de l'étude était de décrire les résultats du traitement à long terme chez les Nigériens infectés par le VIH et d'évaluer l'effet des co-infections par le VHB et le VHC sur la réponse à long terme au traitement antirétroviral (TAR).

**Méthodologie:** Il s'agissait d'une étude rétrospective sur des adultes infectés par le VIH (>18 ans) ayant commencé un traitement antirétroviral consécutivement entre juillet 2004 et décembre 2007, suivis pendant 7 ans (2011 et 2014). Les infections par le VHB et le VHC ont été diagnostiquées par détection de l'antigène de surface sérique de l'hépatite B (AgHBs) et des anticorps anti-VHC (HCVAb) respectivement. La charge virale du VIH et la numération des CD4 ont été surveillées tous les trois mois après le début du TAR, et les résultats du traitement basés sur ceux-ci ont été comparés entre les patients atteints de mono-infection VIH, VIH/VHB, VIH/VHC et VIH/VHB/VHC. Les données cliniques et de laboratoire des patients ont été extraites des bases de données médicales, FileMaker Pro, v 10, saisies dans Microsoft Excel et analysées à l'aide de SPSS version 20.0.

**Résultats:** Un total de 2800 adultes ont été évalués (âge médian de 35,5 ans; 64,2% de femmes), dont 197 (7,0%) étaient co-infectés par le VHB, 53 (1,9%) par le VHC et 15 (0,5%) par le VHB et VHC. Au cours de la période de 7 ans, 369 (13,2%) patients ont été perdus de vue. La reconstitution immunitaire, mesurée par la récupération des CD4, était plus faible dans les co-infections par le VHB et le VHC que dans la mono-infection par le VIH, mais cela n'était pas statistiquement significatif ( $p>0,05$ ). La charge virale VIH de base médiane était de 4,63 log copies / ml pour tous les groupes, ce qui a diminué à un niveau indétectable à une période médiane de 6 mois et le reste pendant toute la durée de l'étude.

**Conclusion:** Cette étude a révélé un échec virologique plus élevé parmi le groupe co-infecté par le VIH / VHC par rapport aux autres groupes. Aucune différence immunologique dans les résultats du traitement TAR entre le VIH mono-infecté et ceux co-infectés par le VHB et le VHC après un suivi de 7 ans. L'augmentation progressive des CD4 s'est avérée être une preuve immunologique de la guérison du corps du VIH, étayée par la baisse de la charge virale au cours de la période de 7 ans.

**Mots clés:** TAR, VIH, VHB, co-infection par le VHC, résultats à long terme

## Introduction:

There are approximately 37.9 million people worldwide living with human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) in 2019 (1). An estimated 1.8 million individuals worldwide became newly infected with HIV and about 5000 new infections per day. HIV remains a dreaded disease that is affecting millions worldwide (2). With decades of evolution of antiretroviral therapy (ART), the impacts of long-term use of ART drugs is largely unknown. To further complicate the issue, co-infections with blood borne hepatitis viruses such as hepatitis B (HBV) and C (HCV) highlights a necessary look into how such cohorts will fare over a long-term treatment.

Highly active antiretroviral therapy (HAART) has increased the life expectancy of HIV-infected individuals who maintain long-term suppression of HIV replication and restore their CD4 counts (3–5). Factors such as the initial HAART regimen, baseline HIV RNA, adherence, and side effects influence the success of achieving long-term HIV RNA suppression, however, it is unclear whether HBV or HCV co-infection affects long-term response to HAART. Chronic hepatitis B (CH-B) occurs in 5–10% of HIV-infected individuals and its long-term influences on the HIV RNA suppression, CD4 recovery, and mortality while on HAART are not

fully characterized. Duda et al., (6) conducted a baseline study for on-going monitoring of the evolution of care delivery over time, evaluating HIV treatment outcomes in relation to site capacity for comprehensive care. However, in spite of the importance of ensuring optimal outcomes, few studies have addressed the capacity of HIV programmes to deliver comprehensive care. This study sought to describe such capacity in a developing country, Nigeria, as a model.

## Materials and method:

### Study setting, design and population

This was a retrospective comparison study, carried out at the Centre for Human Virology and Genomics (CHVG) of the Nigerian Institute of Medical Research (NIMR), Lagos. The Federal Government of Nigeria initiated an antiretroviral drug access programme in 2002, and NIMR was selected as one of the 25 centres. NIMR currently provides comprehensive HIV care, treatment and support for over 16,000 individuals. Majority (75%) of them are from Lagos and Ogun States, while the rest are from neighboring States. The demographics collected included marital status, education, occupation and risk factors.

CHVG is a national reference laboratory for HIV established in 2001, and it was one of

the first national centers that benefited from the US Government President's Emergency Plan for AIDS Relief (PEPFAR) fund. CHVG implements quality management system certified by the International Organization for Standardization (ISO) 9001:2008. The CHVG laboratory was accredited to ISO 15189:2012 by the South African National Accreditation System (SANAS) in 2018 within the scope of molecular diagnostics, chemistry and serology, and was recently listed by WHO as a pre-qualification laboratory.

The study population consisted of HIV-infected confirmed adults who had visited the Clinical Sciences Department (CSD) for medical consultation and the CHVG for laboratory tests, from July 2004 to December 2007, and followed up for 7 years.

#### **Ethical considerations**

Informed consent was obtained in writing from all patients in accordance with the World Medical Assembly (WMA) Declaration of Helsinki, and in accordance with the International Council for Harmonisation (ICH) guideline for Good Clinical Practice (6<sup>th</sup> revision, 2008). The medical ethics committee for research in humans also called the Institutional Review Board of NIMR approved the study protocol.

#### **Clinical and pharmacy follow up visits**

Based on clinic appointments, regular quarterly visits were made to NIMR HIV clinic by the patients in order to have consultations with the medical doctors in charge. Reports on any clinical presentations while on ARVs, adverse effects and other health complaints were noted. Once completed, patients were issued requests for both laboratory visits and drug re-fills from the pharmacy section of the clinic.

#### **Laboratory analyses**

Venous blood samples (~8 ml) for estimations of HIV viral load, CD4 count, clinical chemistry, and haematology (not reported in this study) were obtained by means of vacutainer from each patient, and put into potassium ethylene diamine tetra acetate (K<sup>+</sup> EDTA) bottles. All samples (except for the CD4 count and haematology) were centrifuged at room temperature at 3500 rpm for 10 min within 24 hours of collection. The plasma was then separated and stored at -70°C until analyzed.

HIV was confirmed by Enzyme Linked Immunosorbent Assay (ELISA) method (Gen screen™ Ultra HIV Ag-Ab, Bio-Rad, Marnes-la-Coquette, France), while HBV infection was diagnosed by detection of hepatitis B surface antigen (HBsAg) with Monolisa HBsAg Ultra3 (BioRad Hercules, CA, USA). HCV diagnosis was

made by antibody (HCVAb) detection using Dia Pro Diagnostic Bioprobes, srl, (Milan, Italy).

HIV viral load (VL) was estimated at 3-month intervals by reverse transcription-PCR assay using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v.2.0 test kits (Roche Diagnostics, Branchburg, USA) on the COBAS AmpliPrep, TaqMan48 and 96 analyzers. One milliliter of blood plasma was pipetted into sample tubes of the instrument, and the process divided into three major steps (all automated); specimen preparation, reverse transcription, and simultaneous PCR amplification and detection of target RNA. The assay takes about five and a half hours. The limit of detection/dynamic range of the assay is 20-100,000,000 IU/ml. The median HIV-1 viral load of the 4 categories of patients at each laboratory visit were determined and plotted on a chart.

The CD4 count assay was analyzed at baseline and 3-month intervals using the CY-S-3022 CyFlow® Counter instrument and reagents (Sysmex Partec GmbH, Gorlitz, Germany). Briefly, EDTA whole-blood sample (20 µl) was mixed with antibody conjugated to a fluorochrome in a 1:1 ratio. After a fixed incubation time, the buffer was added and mixture analyzed on the flow cytometer. The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected, while a blood sample is running through the instrument. The concentration of detected cell population was calculated by the integrated software. The median CD4 counts of the 4 categories of patients at each laboratory visit were determined and plotted on a chart.

#### **Data abstraction and statistical analysis**

Data were abstracted from records of adult patients (>18 years) who had laboratory results, clinical information, and drug intake combinations from the CSD and CHVG medical databases, FileMaker Pro, version 10. The patients were sorted into four categories of interest; HIV-1 mono-infected, HIV/HBV co-infected, HIV/HCV co-infected and HIV/HBV/HCV tri-infected groups. Data collected from each patient included age, gender, marital status, education, occupation, height, weight, risk factors, treatment regime combination, first line/second line, HIV viral load, CD4 count, serology status of HIV, hepatitis B and C, and clinical conditions.

Abstracted data were entered into Microsoft Excel 2010 (de-linked and cleaned before analysis) and analysed using the Statistical Package for Social Sciences (SPSS) version 20.0.

## Results:

### Demographics of study population:

A total of 2,800 patients were enrolled within the study period. The median age of the study participants was 35.5 (IQR 25 - 49) years. The majority of the study population were HIV mono-infected with 2,535 (90.5%) patients, followed by HIV/HBV co-infected, 197 (7.0%); HIV/HCV co-infected, 53 (1.9%), while the HIV/HBV/HCV triple infected were only 15 (0.5%). Majority (61.6%) were married, 41.1% had at least a secondary school education, while 63.8% had income generating jobs. The demographics of the study population and risk factors associated with HBV and HCV co-infections are shown in Table 1.

### CD4 cell count:

Three monthly CD4 values of the HIV mono-infected, HIV/HBV and HIV/HCV co-infected, and the HIV/HBV/HCV triple infected patients increased from baseline (month 0) to the 84<sup>th</sup> month (Fig 1). The median  $\pm$  SD CD4 values at the baseline were 221 $\pm$ 34, 184 $\pm$ 22, 222 $\pm$ 28 and 135 $\pm$ 24 cells/ $\mu$ L for the HIV mono-infected, HIV/HBV and HIV/HCV co-infected and HIV/HBV/HCV triple infected patients respectively.

tively. At the end of the study (84<sup>th</sup> month), the median  $\pm$  SD CD4 T cell values had increased to 583 $\pm$ 34, 528 $\pm$ 32, 531 $\pm$ 56 and 549 $\pm$ 19 cells/ $\mu$ L for the HIV mono-infected, HIV/HBV and HIV/HCV co-infected, and HIV/HBV/HCV triple infected patients respectively.

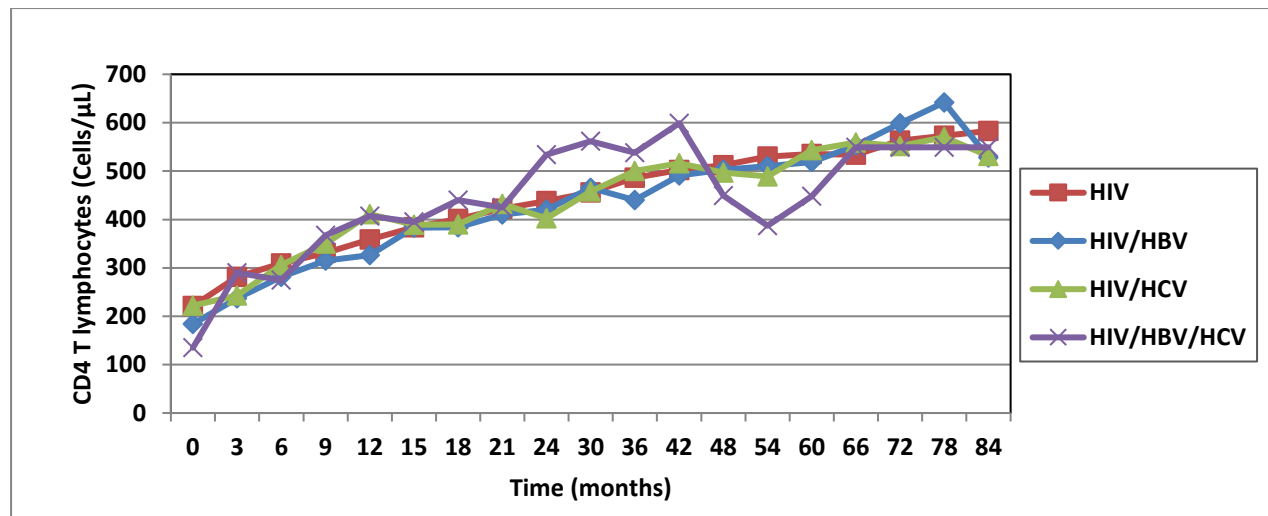
### HIV-1 viral load:

Three monthly viral load values of the HIV mono-infected, HIV/HBV and HIV/HCV co-infected and the HIV/HBV/HCV triple infected patients reduced from baseline (month 0) to the 84<sup>th</sup> month (Fig 2). The median  $\pm$  SD HIV-1 viral load at baseline were 252,285  $\pm$ 3921 (5.4 log), 286,534  $\pm$ 358 (5.4 log), 206,363  $\pm$ 1772 (5.3 log) and 84,480  $\pm$ 1879 (4.9 log) RNA copies/mL for the HIV mono-infected, HIV/HBV and HIV/HCV co-infected, and HIV/HBV/HCV triple infected patients respectively. At the end of the study period, viral load values had dropped to undetectable level (0 RNA copies/ml) for all the patient groups. The viral load values showed a gradual drop of viral titer lingering to the 24<sup>th</sup> month before 'not detected' was achieved. The median baseline HIV viral load of 4.63 RNA log copies/ml for all groups reduced to 'undetected' level at a median time of 6 months, and remained so for the study duration.

Table 1: Demographics of HIV patients on long term HAART and risk factors for HBV and HCV co-infections in Lagos, Nigeria

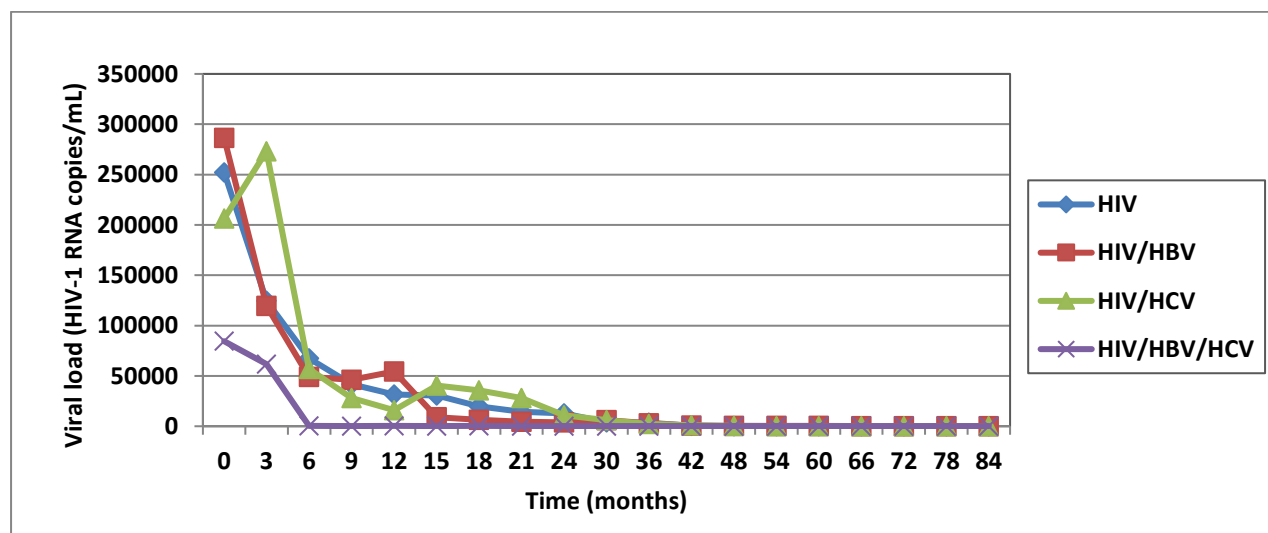
Characteristics	Total (%) (n=2800)	HIV (%) (n=2535)	HIV/HBV (%) (n=197)	HIV/HCV (%) (n=53)	HIV/HBV/HCV (%) (n=15)	p value
<b>Marital status</b>						
Married	1727 (61.6)	1577 (62.1)	109 (55.3)	32 (60.4)	8 (53.3)	0.34
Single	641 (22.8)	564 (22.2)	57 (28.9)	16 (30.2)	4 (26.7)	0.64
Widowed	284 (10.1)	259 (10.2)	19 (9.7)	5 (9.4)	1 (6.7)	0.55
Separated	84 (2.9)	72 (2.7)	10 (5.1)		2 (13.3)	0.78
Divorced	65 (2.3)	63 (2.4)	2 (1.0)			0.87
<b>Education</b>						
Tertiary	806 (28.8)	750 (29.5)	44 (22.3)	12 (25.0)	-	-
Secondary	1151 (41.1)	1031 (40.7)	88 (44.6)	25 (52.1)	7 (50.0)	-
Primary	534 (19.1)	468 (18.4)	49 (24.8)	10 (20.8)	7 (50.0)	-
None	115 (4.1)	107 (4.2)	-	-	-	-
Not indicated	174 (6.8)	174 (6.8)	-	-	-	-
<b>Occupation</b>						
Income generating	1828 (65.2)	1619 (63.8)	156 (79.2)	39 (73.6)	14 (93.3)	-
Non-income generating	816 (29.1)	762 (30.0)	40 (20.3)	13 (24.5)	1 (6.7)	-
Not indicated	156 (5.6)	154 (6.1)	1 (0.5)	1 (1.9)	-	-
<b>Risk factors</b>						
Heterosexual	2210 (78.9)	1996 (78.7)	161 (81.7)	40 (75.4)	13 (86.7)	-
MSM	45 (1.6)	43 (1.7)	1 (0.5)	1 (1.9)	-	-
Transfusion	106 (3.8)	94 (3.7)	8 (4.0)	4 (7.5)	-	-
Unknown	310 (11.1)	277 (10.9)	24 (12.2)	7 (13.2)	2 (13.3)	-
MTCT	2 (0.07)	1 (0.04)	1 (0.5)	-	-	-
IVDU	1 (0.035)	1 (0.04)	-	-	-	-
Heterosexual/Transfusion	34 (1.2)	31 (1.2)	2 (1.0)	1 (1.9)	-	-
Heterosexual/MSM	1 (0.035)	1 (0.04)	-	-	-	-
Heterosexual/IVDU	1 (0.035)	1 (0.04)	-	-	-	-
Heterosexual/unknown	5 (0.2)	5 (0.2)	-	-	-	-

MSM = men who have sex with men; IVDU = intravenous drug user; MTCT = mother-to-child transmission



HIV = Human immunodeficiency virus; HBV = Hepatitis B virus; HCV = Hepatitis C virus

Fig 1: Profile of median CD4 cell count of the four categories of patients during the 7-year period



HIV = Human immunodeficiency virus; HBV = Hepatitis B virus; HCV = Hepatitis C virus  
Gradual decline was observed in the four patient categories that leveled at the 24<sup>th</sup> month

Fig 2: Profile of median HIV viral load of the four categories of patients during the 7-year period

#### Clinical assessment:

Majority (88%, n=2231) of HIV mono-infected patients were clinically stable (with no medical complaints) during the period of assessment, 0.9% (n=24) developed virologic failure, and 55 (2.2%) developed pulmonary tuberculosis. In addition, HIV/HBV, HIV/HCV and HIV/HBV/HCV groups all recorded clinical

stability at 88%, 84.9% and 80% respectively, (as shown in Table 2). During the 7-year period, 335 (11.9%) patients were lost to follow-up or may have died. Immune reconstitution (CD4 recovery) was lower in both HIV/HBV and HIV/HCV co-infected patients, but this difference was not statistically significant ( $p>0.05$ ).



Table 2: Clinical assessment of the HIV patients with HBV and HCV co-infections on long term HAART during a 7-year follow-up in Lagos, Nigeria

Clinical assessment	HIV (%)	HIV/HBV (%)	HIV/HCV (%)	HIV/HBV/HCV (%)	p value
Stable	2231 (88)	175 (88.9)	45 (84.9)	12 (80)	0.763
Virologic failure	24 (0.9)	5 (2.5)	6 (11.3)	0	0.034
Pulmonary tuberculosis	55 (2.2)	7 (3.6)	2 (3.8)	0	0.048
Asymptomatic	4 (0.2)	0	0	0	-
Malaria	50 (1.9)	0	0	1 (6.7)	0.003
Urinary tract infection	11 (0.4)	0	0	0	-
Hypertension	19 (0.8)	1 (0.5)	0	0	0.098
Other complaints	120 (4.7)	5 (2.5)	0	2 (13.3)	0.323
Pruritis	11 (0.4)	3 (1.5)	0	0	0.569
Elevated ALT	10 (0.4)	1 (0.5)	0	0	0.762
Loss to follow up	296 (10.5)	27 (0.96)	9 (0.32)	3 (0.11)	0.006

ALT = Alanine Transaminase

## Discussion:

Patient monitoring is an arduous task, involving skilled manpower, adequate resources, dedicated and disciplined health practitioners and other support staff. Even with all these in place, some patients would inevitably be lost. However, on the bright side, a far larger proportion is maintained in care, and stay healthy despite the burden of daily drug intake. The present study showed a larger proportion was virologically suppressed after 7 years of anti-retroviral therapy. The instance of co-infections of either HBV or HCV or both did not influence viral load decline.

In this study, a large proportion were married (62.1% in the HIV-mono-infected, 55.3% in the HIV/HBV, 60.4% in the HIV/HCV and 53.3% in the HIV/HBV/HCV triple infected groups), followed by singles at 22.2%. In terms of education, about 40% had at least secondary school education across all the study categories, and a third of the population had tertiary education, while very few (4.2%) had no education at all. Majority (>63.8%) of the study population were persons with income generating occupation, while an average of 25% did not have paying jobs. In terms of risk factors, about 80% of the population reported being heterosexuals, 1.7% were homosexuals, 3.7% had had blood transfusion, and 10.9% had no known viral risk factors.

From the literature, it has been reported that once HAART intake is initiated, the natural progression of hepatitis B or C changes. Several

authors have documented that HIV impacts the progression of HCV and increases the likelihood of subsequent liver damage (7,8). The main concerns regarding HAART treatment on co-infected persons are the effects a restored immune response have on the liver and delayed CD4 recovery (9). Clinically this study reports that a large percentage of patients are stable on HAART for the four groups. Sadly, virologic failure was observed more in the HIV/HCV co-infected group (11.3%) than the HIV/HBV (2.5%) and HIV (0.9%) mono-infected groups ( $p=0.034$ ). This is comprehensible due to co-infection. Proponents of reduced monitoring of CD4 and viral load markers refer to a study in Uganda and Zimbabwe on children receiving first-line ART without viral load or CD4 monitoring who had 'not detected' values over 4 years (10), dispelling the need for constant viral load monitoring.

Our study corroborates a Latin American study which reported that despite advanced HIV disease and the use of antiretrovirals, a large fraction of early HAART initiators in the study cohort were alive and in care, with sustained virologic suppression and progressive immune recovery (11).

## Conclusion:

Our study showed significant difference in virologic failure between the HIV/HCV co-infected patients than other patient groups, but no significant difference in the immunological features following ART treatment between HIV

mono-infected patients and those co-infected with HBV and HCV after 7 years of follow-up. The gradual rise in CD4 count was found to be an immunological evidence of the patient body's recovery from the damage inflicted by HIV. This was buttressed by drop in the viral load over the 7-year period.

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## Original Article

## Open Access

## Comparative evaluation of hydrophilic bases for improved delivery of Benzoic acid and Salicylic acid in antimicrobial ointment

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### Abstract:

**Background:** Benzoic acid (BA) and salicylic acid (SA) combined are used as choice topical treatment for fungal and bacterial infections but their delivery is affected by ointment vehicle, among other factors. With aim to achieve improved release and antimicrobial activity in ointment formulation of these medicaments through selection of more efficient vehicle(s), this study comparatively evaluated prospective alternatives to the compendium-specified base for delivery potential and relevant physical properties.

**Methodology:** Water-sorption capacity, rheological characteristics and heat-tolerance limits of six hydrophilic bases [hydrous ointment (HO), hydrous sheabutter (HS), neat sheabutter (NS), lanolin anhydrous (LA), lanolin hydrous (LH), and emulsifying ointment (EO)] and their ointment products containing 1, 2, 3, or 6% w/w of BA or SA were determined. Drug delivery propensity of the products was evaluated by agar diffusion colorimetric assay, while their antimicrobial activities were determined by hole-in-plate agar diffusion assay against selected type organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida pseudotropicalis*).

**Results:** BA was released in relatively larger quantity and demonstrated greater antimicrobial activity than SA in most bases. The released quantities of each medicament correlated directly with concentration and antimicrobial activities. The rates and extents of drug release followed a similar trend in different vehicles namely, HO > HS > NS ≥ EO > LA ≈ LH. Water number of base, ointment preparation method, viscosity, or heat-tolerance showed no influence on drug release or antimicrobial activity.

**Conclusion:** HO and HS are better vehicles for delivery of BA and SA in ointment than EO which is the prototypical base.

**Keywords:** Benzoic acid, Salicylic acid, Antimicrobial activity, Ointment base, Delivery factors

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## Évaluation comparative des bases hydrophiles pour une meilleure administration d'acide benzoïque et d'acide salicylique dans une pommade antimicrobienne

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### Abstrait:

**Contexte:** L'acide benzoïque (BA) et l'acide salicylique (SA) combinés sont utilisés comme traitement topique de choix pour les infections fongiques et bactériennes, mais leur administration est affectée par le véhicule de la pommade, entre autres facteurs. Dans le but d'améliorer la libération et l'activité antimicrobienne dans la formulation de pommade de ces médicaments grâce à la sélection de véhicules plus efficaces, cette étude a évalué comparativement des alternatives potentielles à la base spécifiée dans le compendium pour le potentiel d'administration et les propriétés physiques pertinentes.

**Méthodologie:** Capacité de sorption de l'eau, caractéristiques rhéologiques et limites de tolérance à la chaleur de six bases hydrophiles [pommade hydratée (HO), beurre de karité hydratée (HS), beurre de karité pur (NS), lanoline anhydre (LA), lanoline hydratée (LH), et onguent émulsifiant (EO)] et leurs produits de pommade contenant 1, 2, 3 ou 6% p/p de BA ou SA ont été déterminés. La propension à l'administration de médicaments

des produits a été évaluée par un essai colorimétrique de diffusion sur gélose, tandis que leurs activités antimicrobiennes ont été déterminées par un essai de diffusion sur gélose en plaque contre des organismes de type sélectionné (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* et *Candida pseudotropicalis*).

**Résultats:** BA a été libéré en quantité relativement plus grande et a démontré une plus grande activité antimicrobienne que SA dans la plupart des bases. Les quantités libérées de chaque médicament étaient directement liées à la concentration et aux activités antimicrobiennes. Les taux et l'étendue de la libération de médicaments ont suivi une tendance similaire dans différents véhicules, à savoir  $HO > HS > NS \geq EO > LA \approx LH$ . Le nombre d'eau de base, la méthode de préparation de la pommade, la viscosité ou la tolérance à la chaleur n'ont montré aucune influence sur la libération du médicament ou l'activité antimicrobienne.

**Conclusion:** HO et HS sont de meilleurs véhicules pour la livraison de BA et SA en onguent que EO qui est la base prototypique.

**Mots-clés:** acide benzoïque, acide salicylique, activité antimicrobienne, base de pommade, facteurs de délivrance

## Introduction:

Ointments are semisolid pharmaceutical dosage forms designed for application to human skin or to the surface of the eye or used nasally, vaginally or rectally for topical effect. Most ointments are used for the effects of the therapeutic agents they contain. Non-medicated ointments, on the other hand, are used for physical effects they provide on skin as protectants, emollients or lubricants. They are known as ointment bases and serve in medicated ointments as vehicles (carriers) for the therapeutic agents (1).

Benzoic acid is a crystalline, white or colourless solid organic compound (empirical formula:  $C_6H_5CO_2H$ ), slightly soluble in water (3.44g/L at 25°C). It is the simplest aromatic carboxylic acid also known as phenylcarboxylic acid, phenylmethanoic acid, benzene carboxylic acid, or carboxybenzene (2). Benzoic acid as well as its salts and esters called benzoates possess antibacterial and antifungal properties and are included as ingredients of cosmetic products, used as preservative in foods and pharmaceutical preparations as preservative or as antiseptic agents. Salicylic acid, on the other hand, is a colourless or white crystalline, aromatic organic solid compound with empirical formula,  $C_6H_4(OH)COOH$ , where the hydroxyl group is ortho to the carboxyl group. Salicylic acid is a lipophilic monohydroxybenzoic acid, that is also known as 2-hydroxybenzoic acid. It is a poorly water-soluble phenolic acid (solubility: 2g/L at 20°C) (3). Salts and esters of salicylic acid are called salicylates and have medicinal (analgesic, anti-inflammatory) value. Salicylic acid, as a medication, is commonly used topically (in ointment or cream) for its keratolytic, bacteriostatic and fungicidal properties, to treat warts, psoriasis, acne, ringworm, dandruff, and ichthyosis in pharmaceutical or skincare products (4).

The combined use of benzoic acid and salicylic acid in ointment preparation at 6 and 3% concentrations respectively, known as Whitfield or compound benzoic acid ointment, was on the World Health Organization (WHO)

list of essential medicines until the 16<sup>th</sup> update of 2009 (5,6). Its removal from the 2011 list onward was not attributed to any safety reason or untoward effect but to availability of competitive alternative (7). Earlier, there had been a suggestion to enhance the release of Whitfield ointment's medicaments by the use of a natural base plus surfactants as an alternative to emulsifying ointment, the vehicle recommended by official compendium (8).

The base used to prepare a semisolid medication exerts considerable control over the release and hence the therapeutic action of the medicament it carries. Two general approaches are used for topical preparations to maximize absorption of the active ingredient from the vehicle; inclusion of an agent that affects the barrier function of the epidermis (9), or employment and development of a vehicle with physical characteristics that favour diffusion of the drug from the vehicle to the skin using an experimental procedure that measures drug release from the vehicle, from which the most suitable base is selected. In the latter approach however, inclusion of surfactant into semisolid base could contribute multifarious effects; sometimes improving drug release (10,11), reducing release (12,13), or not affecting drug release at all (14).

Hydrophilic ointment bases are those permitting addition of water (or aqueous solutions) due to their water-miscibility or water absorption capacity. Their advantages over hydrophobic bases include being water-washable (water-removable), compatible with normal skin functions and exhibiting more efficient or rapid drug delivery properties even with exclusion of surfactant (1,15). The aim of this study, therefore, was to comparatively evaluate five hydrophilic ointment bases as prospective alternatives for improved delivery of benzoic acid and salicylic acid from dispersion ointment formulation, and correlate the findings with concurrently determined antimicrobial activities of the medicaments in order to elucidate the factors

that may contribute to enhanced drug delivery function.

## Materials and method:

### Study setting

The study was carried out at the postgraduate pharmaceutical technology and pharmaceutical microbiology laboratories of the Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

### Materials

Materials used in the study were Benzoic acid (Fig 1A) and salicylic acid (Fig 1B) (May and Baker, Dagenham, England) each screened through 180  $\mu\text{m}$ -aperture (mesh no. 85) standard sieve (Okhard Machine Tools Co. Ltd, Lagos Nigeria); emulsifying wax (Evans Medical Ltd. Liverpool); liquid, white soft, and hard paraffin (William

Ransom and Sons Plc. England); lanolin anhydrous, and wool alcohols (BDH, Poole, England).

Hydrophilic ointment bases studied were hydrous ointment (HO), hydrous sheabutter (HS), neat sheabutter (NS), lanolin anhydrous (LA), lanolin hydrous (LH), and emulsifying ointment (EO), having composition as given in Table 1. Neat sheabutter was obtained by hot (60°C) filtration processing of natural sheabutter (16) procured at Shaki market, Oyo State, Nigeria, while lanolin anhydrous was as obtained from its manufacturer. Emulsifying ointment BP and hydrous ointment BP were prepared according to the compendium guidelines (17). Microbiological culture media used were Mueller-Hinton (MH), Nutrient agar (NA), and Sabouraud's Dextrose agar (Rapid Labs Ltd, Colchester Essex, UK) which were prepared according to the manufacturers' instructions.

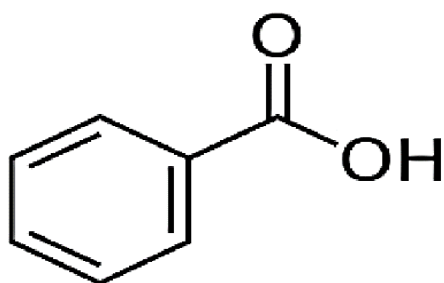


Figure 1A: Chemical structure of benzoic acid  
molecular weight: 122.12 g/mol

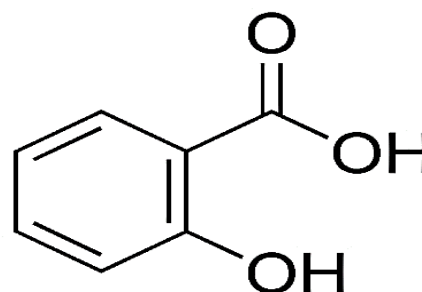


Figure 1B: Chemical structure of salicylic acid  
molecular weight: 138.12 g/mol

Table 1: Composition of ointment bases and of Whitfield ointment

Ingredients	Name of Ointment Base/ Ingredient Concentrations (%w/w)				Whitfield Ointment BP composition (%w/w)
	Hydrous ointment BP	Emulsifying ointment BP	Lanolin hydrous	Hydrous sheabutter	
Liquid paraffin	30	20	–	–	18.2
Hard paraffin	12	–	–	–	–
White soft paraffin	5	50	–	–	45.5
Wool alcohols	3	–	–	–	–
Emulsifying wax	–	30	–	–	27.3
Lanolin anhydrous	–	–	70	–	–
Neat sheabutter	–	–	–	70	–
Water (purified)	50	–	30	30	–
Benzoic acid	–	–	–	–	6.0
Salicylic acid	–	–	–	–	3.0

–: ingredient not contained in ointment

### Determination of water number of ointment bases

The water number (water-sorption capacity) of the six ointment bases (10g samples) was determined at the ambient temperature ( $30\pm 2^{\circ}\text{C}$ ) using the pharmacopeial method described for hydrous wool fat (17) and expressed conventionally as the volume of water (mL) absorbed per 100g of base.

### Preparation of medicated ointments

Two batches (100g each) of medicated ointment preparations were made with each ointment base using two different methods; levigation and fusion. In the levigation method, the medicament [benzoic acid at 1, 2, 3, or 6%; and salicylic acid at 1, 2, or 3% (w/w) concentrations] was incorporated by mixing into the ointment base with spatula on glazed porcelain tile at the ambient temperature ( $30\pm 2^{\circ}\text{C}$ ) until a uniform preparation was attained. In the fusion method, however, mild heat ( $\leq 50^{\circ}\text{C}$ ) was applied to soften or melt the base, which facilitated subsequent incorporation of the medicament, followed by constant stirring of the preparation while allowing it to cool until congealed (18). The medicated ointment samples, packaged (20g aliquots) in screw-cap (airtight) ointment jars were subsequently assessed for their physical stability and release properties as follows;

### Viscometric testing of ointments

Viscosity measurements were carried out on the bland and medicated ointment samples (20g each) at the ambient temperature ( $30\pm 2^{\circ}\text{C}$ ) using a Rion VT-04F viscometer, spindle no. 2 (Rion Co. Ltd, Tokyo Japan). The yield point value (initial peak) and viscosity of each sample were determined for 30s. Any physical consistency changes in the sample under test were noted.

### Heat-tolerance limit testing of ointments

Softening and melting temperatures of bland bases and of completed medicated ointments, and the congealing temperature values on cooling to ambient temperatures after melting, were determined using the method for suppository bases described by Adegbeye and Itiola (19). Softening point was the temperature at which the sample began to liquefy, while melting point was the temperature of complete liquefaction. The mean and standard deviation (SD) of four determinations were recorded.

### Determination of *in vitro* drug-release from ointments

The drug release propensity of medicated ointments was determined by the agar diffusion colorimetric assay earlier described (20,21) and modified as follows; To 20mL of

1% (w/v) bacteriological agar gel previously melted and cooled to  $45^{\circ}\text{C}$  was added and mixed gently 0.5mL of appropriate colour-indicator solution, and poured into clean 90-mm diameter Petri dishes and allowed to set. Five percent (w/v) aqueous solution of bromocresol green and ferric chloride served as the appropriate indicators for testing ointments containing benzoic acid and salicylic acid respectively. After 30 min, three equidistant cups (8.0 mm in diameter) were made into the agar plate using a sterile cork-borer and 0.2mL (approximately 170mg) of medicated ointment sample was extruded from a 5mL glass syringe into each agar cup (triplicate experiments), placed such that it made uniform contact with the agar gel.

The plates were incubated at  $37^{\circ}\text{C}$  in aerobic incubator. The diameters of colour-zone changes produced around each cup as the medicament was released and diffused through agar from the ointment sample were measured at predetermined time intervals; 10, 20, and 30 min intervals during the 1<sup>st</sup>, 2<sup>nd</sup>, and until the 6<sup>th</sup> hours respectively, and then hourly, 2-hourly, and 4-hourly intervals until the 10<sup>th</sup>, 16<sup>th</sup>, and 24<sup>th</sup> hours respectively. The area of coloured zones was calculated from the mean diameter of each zone to indicate extent of the medicament released from ointment at the various data-sampling times from which the rate of release was calculated. The release rate of benzoic acid or salicylic acid from each medicated ointment preparation in each base was determined as the slope value in the regression line equation of the plot of diffusion-zone size of the medicament versus the square-root of time (22).

### Determination of antimicrobial activity of the ointment products

#### Test microorganisms and culture conditions

The test organisms used were *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Candida albicans* (ATCC 24433), and *Candida pseudotropicalis* (NCYC 6). The bacterial strains were grown on Nutrient agar slopes at  $37^{\circ}\text{C}$  for 24 hours while the fungal cultures were grown on Sabouraud's Dextrose agar slopes for 48 hours at  $25^{\circ}\text{C}$ . The organisms were maintained on slopes and sub-cultured weekly.

#### Screening for antibacterial and antifungal activities

For antibacterial activity testing, 0.5 mL of 0.5 McFarland standards turbidity suspension (approx.  $1\times 10^8$  CFU/mL) of each bacterial strain was inoculated and spread evenly on the surface of over-dried Mueller-

Hinton (MH) agar plates while for antifungal activity testing, 48 h surface culture of each fungal strain on Sabouraud's Dextrose Agar (SDA) slopes that were washed off and diluted to an inoculum size of  $10^7$  CFU/mL was used. 0.5 mL of this suspension was evenly spread on the surface of over-dried SDA plates.

The hole-in-plate agar diffusion method (23) was used to assess the anti-microbial activities of ointment products containing benzoic acid or salicylic acid (at 1, 2, 3, 4.5, or 6% w/w concentrations of the medicament) made by levigation using different hydrophilic bases against each test organism. A 10g sample of each of the six medicated ointments was made for testing, Whitfield ointment (Table 1) serving as comparator ointment. Three equidistant wells (8 mm diameter each) were cut into each prepared (inoculated) agar plate using a sterile cork-borer, and approximately 170mg of medicated ointment sample for testing (0.2mL extruded from a 5mL glass syringe) was introduced into each well for each ointment (test was done in triplicate). The plates were refrigerated (4°C) for 1 hour to allow for diffusion of incorporated medicament and then incubated at 37°C aerobically for 24 hr for bacteria and 25°C for 48 h for fungi. The diameters of inhibition zones (in mm) were measured. Bland ointment bases were evaluated alongside the medicated ointments as negative control.

Following the same testing procedure, the antimicrobial activity of aqueous solution of benzoic acid and salicylic acid, each prepared in 50% v/v aqueous methanol as solvent (at 0.5, 1, 2, or 4% w/v concentrations respectively as positive controls), was also evaluated against each bacterial and fungal strains. A 100µL sample of each test solution was placed similarly (in triplicate) in equidistant wells of appropriately prepared agar plates for testing. The 50% aqueous methanol solvent alone was evaluated as negative control.

#### Data analysis

The minimum inhibitory concentration (MIC) of benzoic acid and salicylic acid against each test organism was determined by linear regression analysis of the inhibition zone sizes produced by each medicament over graded concentrations in aqueous methanol solution, plotted against the logarithm of concentration (24). The extents of release of

the medicaments from different ointment bases in 24 h, and correlation of the extents against medicament concentration, were evaluated for significance using *t* test. Factors influencing the release of medicament from the bases were investigated by Spearman's rank order correlation of the release rate data compared against each of four factors namely; the preparation method, viscosity under shear of the 3% medicated ointments batch, the water number, and softening point of the bases. Significance was accepted at  $p < 0.05$  level.

## Results:

#### Water number and viscosity of ointments

Table 2 shows the water number of non-medicated bases as well as viscosity of the bland and medicated ointments. Among the bland bases, emulsifying ointment (EO) demonstrated the highest water-sorption capacity, with unlimited absorption upon step wise addition of water until it converted, in the process, to fluid oil-in-water emulsion. Lanolin anhydrous (LA) exhibited the next highest water number (205.0mL/100g), while hydrous sheabutter (HS) showed the least water number (10.5mL/100g).

All the six ointment bases exhibited thixotropic (Bingham plastic) character with different yield point stress values: hydrous ointment (HO) 50; hydrous sheabutter (HS) 60; neat sheabutter (NS) 150; lanolin anhydrous (LA) 75; lanolin hydrous (LH) 80; and emulsifying ointment (EO) 110 Pa.s, followed by decline to a constant viscosity over 15–20s of measurement (Fig 2), becoming steady on the constant apparent viscosity values of hydrous ointment (HO) 12, hydrous sheabutter (HS) 10, neat sheabutter (NS) 45, lanolin anhydrous (LA) 40, lanolin hydrous (LH) 60, and emulsifying ointment (EO) 60 Pa.s (Table 2).

Incorporation of the medicaments (benzoic acid or salicylic acid) into the bases mostly resulted in ointment products of lower viscosity than the bland base. Exception to this general observation occurred with HO and HS, medicated ointment products of which exhibited higher viscosities than those of the respective bland vehicles. However, on occasions, HS base or its medicated product suffered texture-breakdown under levigation or fusion processing, accompanied by marked reduction in viscosity (Table 2).



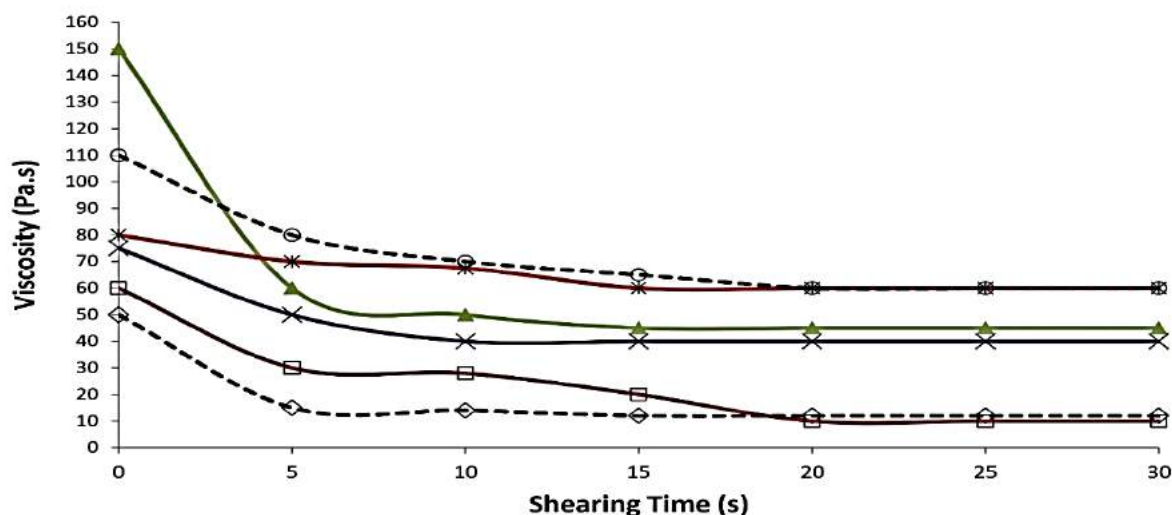


Figure 2: Viscosity Profile of Ointment Bases

—◆— Hydrous Ointment (HO)      —□— Hydrous Sheabutter (HS)      —▲— Neat Sheabutter (NS)  
 —×— Lanolin Anhydrous (LA)      —\*— Lanolin Hydrous (LH)      —○— Emulsifying Ointment (EO)

Table 2: Water number and viscosity of ointments

Ointment Base/ Its Water Number (mL/100g)*	Concentration of Medicament (%w/w)	Medicament/ Preparation Method/ Apparent Viscosity of Ointment (Pa.s)			
		Benzoic acid		Salicylic acid	
		Levigation	Fusion	Levigation	Fusion
Hydrous Ointment BP 107.5 ± 2.9	Ointment base	0	*12		
	Medicated Ointment	1	10	13	20
		2	15	13	20
		3	15	13	20
		6	15	—	—
Hydrous Sheabutter 10.5 ± 1.0	Ointment base	0	*10		
	Medicated Ointment	1	<sup>b</sup> 10	70	40
		2	<sup>b</sup> 10	<sup>b</sup> 10	40
		3	<sup>b</sup> 10	<sup>b</sup> 10	<sup>b</sup> 10
		6	<sup>b</sup> 10	—	—
Neat Sheabutter 117.5 ± 2.9	Ointment base	0	*45		
	Medicated Ointment	1	40	40	40
		2	30	40	40
		3	30	35	35
		6	25	—	—
Lanolin Anhydrous 205.0 ± 4.1	Ointment base	0	*40		
	Medicated Ointment	1	35	35	40
		2	30	30	40
		3	28	23	35
		6	25	—	—
Lanolin Hydrous 170.0 ± 9.1	Ointment base	0	*60		
	Medicated Ointment	1	45	50	55
		2	40	50	25
		3	40	40	15
		6	30	—	—
Emulsifying Ointment BP Indefinite**	Ointment base	0	*60		
	Medicated Ointment	1	50	30	40
		2	40	30	35
		3	35	25	35
		6	35	—	—

\*: Water number values express the Mean ± SD of quadruplicate test results.

\*: Value with this superscript is the apparent viscosity (under shear; Pa.s) of the ointment base in the same row.

<sup>b</sup>10: Processing of hydrous sheabutter base occasionally caused weakness of its texture and water-leaching ("bleeding") accompanied by viscosity-drop to  $\approx 10$  Pa.s.

\*\*\*: Emulsifying ointment absorbed unlimited water, eventually becoming fluid emulsion.

—: Physical characteristics study did not include the 6 %w/w salicylic acid-in-ointment samples.

### Heat-stress vulnerability of ointments

Heat-tolerance thresholds of the ointment bases varied, ranging from the 37°C softening point of neat sheabutter (NS) and of emulsifying ointment (EO) to 56°C, the melting point of lanolin hydrous (LH). But their softening points were very close to their respective congealing point values (within  $\pm 1^\circ\text{C}$ ) except for hydrous sheabutter (HS)

(Table 3).

Incorporation of medicament into each base caused a drop to lower values of heat-tolerance limit of the resultant ointment products; except lanolin anhydrous (LA) with 36°C softening point of the bland base against (nearly the same) 35 or 36°C softening point value of its medicated ointments, and 39°C melting point of the non-med-

Table 3: Heat-stress vulnerability of ointments

Ointment Base	Vulnerability Parameters	Vulnerability Point for Ointment Base (°C) <sup>a</sup>	Ointment Preparation Method/ Active Principle (3 %w/w)/ Vulnerability Point (°C) <sup>a</sup>			
			Levigation		Fusion	
			Benzoic acid		Salicylic acid	
Hydrous ointment BP	Softening	41 ± 0.7	34 ± 0.3	34 ± 0.5	35 ± 0.3	35 ± 0.3
	Congeaing	42 ± 0.7	38 ± 0.5	38 ± 0.3	39 ± 0.3	37 ± 0.3
	Melting	47 ± 0.3	39 ± 0.5	39 ± 0.3	43 ± 0.5	41 ± 0.3
Hydrous Sheabutter	Softening	<sup>u</sup> 38 ± 0.4	<sup>u</sup> 33 ± 0.5	<sup>u</sup> 31 ± 0.3	<sup>u</sup> 32 ± 0.3	<sup>u</sup> 32 ± 0.3
	Congeaing	<sup>u</sup> 28 ± 0.4	<sup>u</sup> 23 ± 0.5	<sup>u</sup> 22 ± 0.5	<sup>u</sup> 23 ± 0.3	<sup>u</sup> 22 ± 0.3
	Melting	42 ± 0.5	34 ± 0.5	33 ± 0.3	37 ± 0.5	36 ± 0.3
Neat Sheabutter	Softening	<sup>s</sup> 37 ± 0.3	<sup>u</sup> 32 ± 0.2	<sup>u</sup> 32 ± 0.2	<sup>u</sup> 33 ± 0.3	<sup>u</sup> 33 ± 0.3
	Congeaing	<sup>u</sup> 37 ± 0.3	<sup>u</sup> 26 ± 0.2	<sup>u</sup> 24 ± 0.2	<sup>u</sup> 23 ± 0.3	<sup>u</sup> 22 ± 0.3
	Melting	39 ± 0.3	33 ± 0.2	35 ± 0.2	37 ± 0.3	37 ± 0.3
Lanolin Anhydrous	Softening	36 ± 0.3	36 ± 0.3	35 ± 0.3	35 ± 0.3	36 ± 0.3
	Congeaing	37 ± 0.3	35 ± 0.3	35 ± 0.3	36 ± 0.3	35 ± 0.3
	Melting	39 ± 0.3	42 ± 0.5	42 ± 0.5	42 ± 0.3	41 ± 0.3
Lanolin Hydrous	Softening	45 ± 0.7	37 ± 0.5	35 ± 0.3	37 ± 0.3	37 ± 0.3
	Congeaing	47 ± 0.5	36 ± 0.3	34 ± 0.3	35 ± 0.3	36 ± 0.3
	Melting	56 ± 0.8	42 ± 0.5	41 ± 0.5	46 ± 0.5	46 ± 0.5
Emulsifying Ointment BP	Softening	37 ± 0.3	34 ± 0.3	34 ± 0.3	34 ± 0.5	35 ± 0.5
	Congeaing	39 ± 0.3	36 ± 0.3	36 ± 0.5	39 ± 0.3	39 ± 0.5
	Melting	43 ± 0.3	38 ± 0.7	41 ± 0.5	39 ± 0.4	40 ± 0.3

<sup>a</sup>Values represent the mean ± SD<sup>u</sup>Unstable polymorphic base form<sup>s</sup>Stable base form

icated base versus 41-42°C melting point values of its medicated ointments, showing an increase following medicament incorporation (Table 3). Under mild but protracted heating (39-42°C for 10-15 min) required to soften or melt HS or NS base for incorporation of medicament, polymorphic transformation of the base often occurred, resulting in weaker, unstable texture of the base, which gave ointment products of a lower congealing point ( $\leq 26^\circ\text{C}$ ) than that of the precursor base (Table 3).

#### Direct correlation of medicament concentration with release

The amounts of each medicament released in 24 hours from each medicated ointment base were in direct trend with the medicament concentrations; 1, 2, and 3% respectively (Figs 3 and 4). The increase of the amounts of each medicament released in 24 hours with the stepwise increase in medicament concentration in benzoic acid ointments (from 1 to 6% w/w), and in Salicylic acid ointments (from 1 to 3% w/w) was, in each case, significant at  $p < 0.01$ .

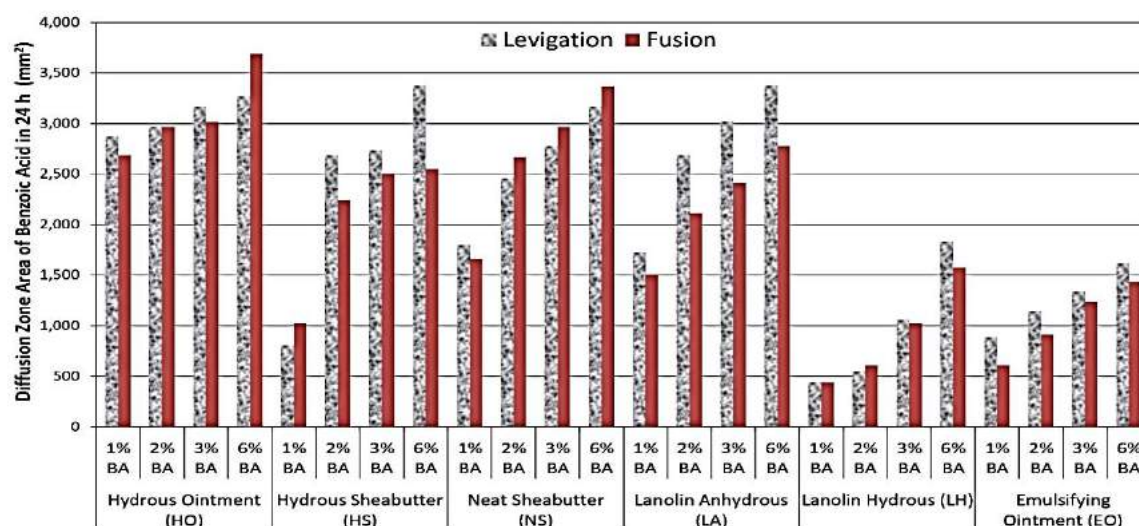


Figure 3: Benzoic Acid (BA) Quantities Released from Hydrophilic Ointment Bases

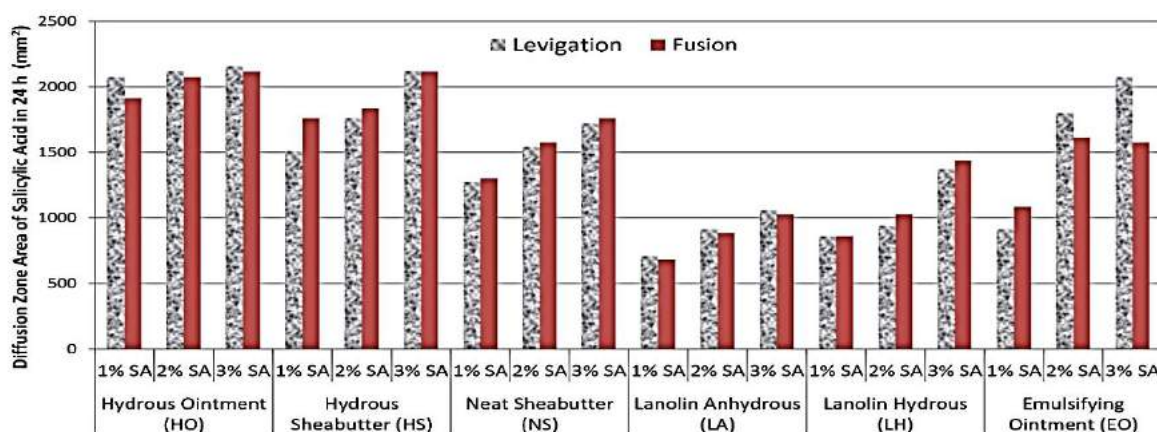


Figure 4: Salicylic Acid (SA) Quantities Released from Hydrophilic Ointment Bases

Table 4: Rates of release of Benzoic acid and Salicylic acid from 3% w/w medicated ointment in different bases

Ointment Base	Medicament/ Preparation Method/ Release Rates (mm <sup>2</sup> min <sup>-½</sup> ) <sup>d</sup>			
	Benzoic Acid		Salicylic Acid	
	Levigation	Fusion	Levigation	Fusion
Hydrous Ointment (HO)	2.32 (0.95)	2.04 (0.96)	1.04 (0.99)	1.06 (0.99)
Hydrous Sheabutter (HS)	2.75 (0.98)	1.86 (0.99)	1.06 (1.00)	0.90 (0.99)
Neat Sheabutter (NS)	2.81 (0.98)	2.78 (0.99)	0.92 (0.97)	0.93 (0.97)
Lanolin Anhydrous (LA)	1.94 (0.97)	1.91 (0.98)	0.58 (0.96)	0.57 (0.95)
Lanolin Hydrous (LH)	0.42 (0.98)	0.54 (0.97)	0.68 (0.98)	0.64 (0.99)
Emulsifying Ointment (EO)	0.58 (0.99)	0.53 (0.95)	0.88 (0.99)	0.69 (0.99)

<sup>d</sup> regression coefficients are shown in parentheses

#### Influence of ointment base on drug release

Benzoic acid was released to significantly greater extents than salicylic acid in 24 h from HO, HS, NS, and LA comparing both medicaments at same concentrations; 1, 2, or 3%, respectively ( $p < 0.01$ ) (Figs 3 and 4). The benzoic acid amounts released from HO, HS, and NS were comparable ( $p > 0.05$ ) but significantly greater than the amounts released from other bases (LA, LH, and EO) in the 24-h period ( $p < 0.01$ ).

Salicylic acid, on the other hand, was released to significantly greater ( $p < 0.01$ ) extents than benzoic acid from LH over 24 h at identical concentrations; 1, 2, and 3%, respectively (Figs 3 and 4). But the extents of release of the two medicaments from EO were not significantly different ( $p > 0.05$ ). The delivery of salicylic acid from five ointment bases occurred in significantly different amounts ( $p < 0.01$ ) in the following descending order; HO > HS > NS > EO > LA but its release from LA and LH was comparable ( $p > 0.05$ ). The trend of the magnitude of rates of release of the medicaments from different ointment vehicles (Table 4) was similar to that found for the extents of release of the medicaments. In summary, the

three ointment bases, HO, HS and NS, demonstrated better release property for both medicaments than EO, LA and LH.

#### Other presumed factors had no influence on drug delivery

Different methods of ointment preparation, point differences in ointments' softening, various viscosity values under shear, and diverse water numbers of the bases did not produce significant observable trend effects on the rates of release of medicament from the medicated ointments studied ( $p > 0.05$ ). No correlation was found between these parameters and the drug release rate data.

#### Antimicrobial activities of Benzoic acid and Salicylic acid in aqueous solution

Aqueous methanol solution of each medicament at 1% w/v and higher concentrations produced clear zones of inhibition against each test organism, while the 50% aqueous methanol solvent alone (negative control) showed no inhibitory activities. Increase of the medicament concentrations from 1 through 4% w/v gave correspondingly larger inhibition zone sizes (Tables 5 and 6).



Table 5: Antimicrobial activities of Benzoic acid in ointment formulation

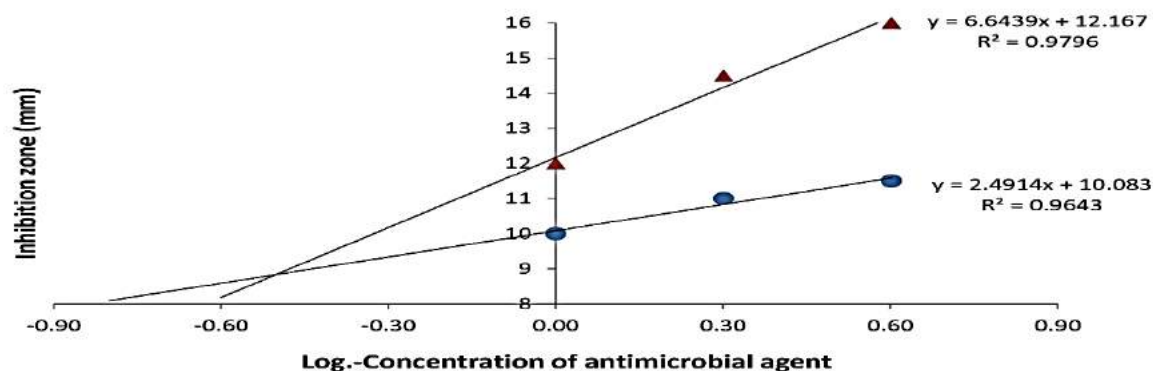
Formulation vehicle	Benzoic acid concentration (% w/v or w/w)	Test organism/Inhibition zone diameter (mm)*					
		<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>C. pseudotropicalis</i>
Hydrous Ointment (HO)	1.0	8.5±0.5	0.0	0.0	0.0	21.5±0.5	20.5±0.5
	2.0	9.5±0.5	9.5±0.5	9±0	0.0	27±3	23±1
	3.0	12±0	10±1	9±0	9±1	30.5±0.5	24.5±0.5
	4.5	13±1	12±2	12±2	10.5±0.5	30.5±0.5	25.5±0.5
	6.0	13±1	15±0	13.5±2.5	12±0	45±0	27.5±0.5
Hydrous Sheabutter (HS)	1.0	0.0	0.0	0.0	0.0	0.0	11±1
	2.0	8.5±0.5	0.0	0.0	0.0	14±0	14±1
	3.0	10.5±1.5	0.0	8.5±0.5	0.0	16±0	13±1
	4.5	12±2	9±1	10±0	0.0	23±1	20±0
	6.0	12.5±1.5	9±1	14.5±0.5	9±1	28.5±0.5	22.5±2.5
Neat Sheabutter (NS)	1.0	9±0	0.0	0.0	0.0	0.0	0.0
	2.0	9±0	0.0	0.0	0.0	9±0	10.5±1.5
	3.0	9±0	0.0	9±1	0.0	14±0	16.5±1.5
	4.5	12.5±0.5	8.5±0.5	10±0	0.0	16±0	19±1
	6.0	14±0	9.5±0.5	10.5±0.5	8.5±0.5	18±0	24.5±0.5
Lanolin Anhydrous (LA)	1.0	0.0	0.0	0.0	0.0	8.5±0.5	9±0
	2.0	8.5±0.5	0.0	0.0	0.0	8.5±0.5	10±0
	3.0	8.5±0.5	8.5±0.5	8.5±0.5	0.0	15±1	12±0
	4.5	9±0	9±0	10±0	0.0	17±1	15.5±0.5
	6.0	9±0	12±1	11.5±0.5	0.0	21±2	19.5±0.5
Lanolin Hydrous (LH)	1.0	0.0	0.0	8.5±0.5	0.0	9.5±1.5	8.5±0.5
	2.0	0.0	0.0	8.5±0.5	0.0	9.5±0.5	9.5±0.5
	3.0	0.0	0.0	8.5±0.5	0.0	11.5±0.5	10.5±0.5
	4.5	0.0	0.0	9±1	0.0	20.5±0.5	18±0
	6.0	11±2	9±0	10.5±0.5	0.0	21±1	24.5±3.5
Whitfield Ointment BP		9±1	12.25±0.25	12.5±0.5	0.0	23±2	22±0.5
<b>Controls:</b>							
50% v/v aqueous methanol (solvent)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.5	9±1	0.0	0.0	0.0	0.0	0.0
Benzoic acid solution in 50% v/v aqueous methanol	1.0	13.5±0.5	10.5±1.5	10±0	11.5±2.5	19±1	28.5±0.5
	2.0	14±2	13±0	11±0	13±0	23.5±3.5	34±0
	4.0	15±1	13.5±0.5	11.5±1.5	15±2	26.5±1.5	34.5±0

\*Data are presented as mean ± range of inhibition zone diameters  
0.0 = Zero value: No inhibition

Table 6: Antimicrobial activities of Salicylic acid in ointment formulation

Formulation vehicle	Salicylic acid concentration (% w/v or w/w)	Test organism/ Inhibition zone diameter (mm)*					
		<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>C. pseudotropicalis</i>
Hydrous Ointment (HO)	1.0	0.0	10±0	0.0	0.0	8.5±0.5	0.0
	2.0	10±1	10.5±1.0	11.25±0.25	0.0	17±0.5	11±0
	3.0	10.25±1.25	11.75±0.75	13.25±0.25	9±0	19.5±0	14.5±0.5
	4.5	12.5±0.5	12.25±1.25	13.75±0.25	11.25±0.25	21.25±0.25	16.25±1.25
	6.0	13.5±0.5	15.75±0.75	15.5±0.5	13.25±1.25	23.5±0.5	17.75±0.75
Hydrous Sheabutter (HS)	1.0	0.0	9±0	0.0	0.0	9±0	0.0
	2.0	9±0	9.75±0.75	9±0	0.0	18.5±0	16±1
	3.0	9.5±0.5	12.25±0.75	10.5±1.5	9±0	23.5±1.0	18±0.5
	4.5	11±1	12.75±1.25	11.75±0.25	12.25±0.25	26±1	19±0.5
	6.0	14±0	16±0	16.75±1.75	13.5±0.5	28.5±1.0	23.25±0.75
Neat Sheabutter (NS)	1.0	0.0	11±1	9.25±1.25	0.0	0.0	0.0
	2.0	8.5±0.5	11.5±0.5	12±0	9.5±1.5	11±2	8.5±0.5
	3.0	10±1	13±0	12.5±0	11.75±0.75	14±1	9.5±0.5
	4.5	10.5±0.5	13.25±0.25	14.75±2.25	12.75±0.25	15.5±0.5	13.75±0.75
	6.0	12.25±0.25	15.25±0.25	16.75±1.75	13±0	17.75±0.25	16±0.5
Lanolin Anhydrous (LA)	1.0	0.0	0.0	0.0	0.0	0.0	0.0
	2.0	0.0	0.0	0.0	0.0	0.0	0.0
	3.0	0.0	0.0	8.5±0.5	0.0	0.0	0.0
	4.5	0.0	0.0	8.5±0.5	0.0	9±0	9±0
	6.0	0.0	0.0	8.5±0.5	0.0	10±1	10±0
Lanolin Hydrous (LH)	1.0	0.0	0.0	0.0	0.0	0.0	0.0
	2.0	0.0	0.0	0.0	0.0	0.0	0.0
	3.0	0.0	0.0	0.0	0.0	0.0	0.0
	4.5	0.0	8.5±0.5	8.5±0.5	0.0	0.0	9±0
	6.0	9±0	8.5±0.5	9.75±0.75	0.0	9±0	9.5±0.5
Whitfield Ointment BP		9±1	12.25±0.25	12.5±0.5	0.0	23±2	22±0.5
<b>Controls:</b>							
50% v/v aqueous methanol (solvent)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.5	8.5±0.5	0.0	0.0	0.0	0.0	0.0
Salicylic acid solution in 50% v/v aqueous methanol	1.0	9±1	8.5±0.5	12±0	8.5±0.5	9±1	9.75±0.25
	2.0	10.75±1.75	9±0	14.5±0.5	10.5±0.5	14±1	16±0
	4.0	15±0	12.5±0.5	16±0	12±1	21.75±1.25	19±1

\*Data are presented as mean ± range of inhibition zone diameters  
0.0 = Zero value: No inhibition



**Figure 5: Regression lines of inhibitory activities of Benzoic acid and Salicylic acid against *E. coli***

● Benzoic acid                      ▲ Salicylic acid  
— Linear (Benzoic acid)              — Linear (Salicylic acid)

**Table 7: Minimum Inhibitory Concentration of Benzoic acid and Salicylic acid against selected organisms**

Organism	Medicament/ Minimum inhibitory concentration (MIC) (mg/mL)	
	Benzoic acid	Salicylic acid
<i>Candida pseudotropicalis</i>	0.072	7.09
<i>Candida albicans</i>	1.25	9.43
<i>Escherichia coli</i>	1.46	2.36
<i>Pseudomonas aeruginosa</i>	2.58	7.94
<i>Staphylococcus aureus</i>	2.70	10.00
<i>Bacillus subtilis</i>	2.28	5.65

Fig 5 illustrates that the logarithm values of the MICs of benzoic acid and salicylic acid against *E. coli*, obtained from regression lines plotted for the medicaments' inhibitory activities, were  $-0.83$  and  $-0.62$  respectively, leading to derivation of the MIC values of  $0.146\%$  and  $0.236\%$  w/v (i.e.  $1.46$  and  $2.36$  mg/mL) respectively. Each MIC value for the medicaments (Table 7) was similarly determined, being the antilogarithm value of the abscissa coordinate (medicament concentration) at the intercept of extrapolated regression line with the horizontal (x-), or log.-concentration axis of the graph where  $y=8.0$ mm signifying the  $8.0$ mm agar-well diameter and which (MIC value) could be alternatively obtained by algebraic derivation from the relevant best-fit line equation (24).

Because the MIC values showed the relative susceptibilities of the test organisms to the antimicrobial activities of the medicaments, they revealed the yeast cells (*C. albicans* and *C. pseudotropicalis*) to be more susceptible than bacterial cells to benzoic acid and that *C. pseudotropicalis* was the more sensitive yeast strain (lower MICs:  $0.072$  and  $7.09$  mg/mL) while *E. coli* was the most susceptible bacterial organism to both benzoic acid and salicylic acid (least MIC

values:  $1.46$  and  $2.36$  mg/mL) but *S. aureus* was the least sensitive bacterium tested, showing the highest MIC values:  $2.70$  and  $10.00$  mg/mL respectively (Table 7).

#### Antimicrobial activities of Benzoic acid and Salicylic acid in ointment products

Each medicament incorporated in different ointment bases at graded concentrations ( $1-6\%$  w/w) exhibited clear inhibition zones of different sizes against the test organisms (Tables 5 and 6), while non-medicated bases (negative controls) showed no inhibitory activities. The medicament concentration, its MIC relative to each test organism and the properties of each base in each ointment preparation all interacted multifariously to determine the antimicrobial activity outcomes, summarized as follows;

The yeast cells were relatively more susceptible than the bacterial cells to the inhibitory action of each medicament in ointment product. *E. coli* was the most sensitive while *P. aeruginosa* was the least sensitive bacteria to the inhibitory action of salicylic acid as medicament in almost all the ointment bases. *P. aeruginosa* was also the least sensitive bacterium to benzoic acid as medicament in all the six ointment vehicles.

However, the relative susceptibility of *E. coli*, *B. subtilis*, and *S. aureus* to benzoic acid-medicated ointments depended on the vehicle employed.

#### **Comparison of drug release results to anti-microbial activities in ointment**

Comparison of delivery propensities of the medicaments in ointment preparations to their antimicrobial activities, in parallel study toward elucidating correlations and contributing factors, gave the following results; two ointment bases, hydrous ointment (HO) and hydrous sheabutter (HS) were prominent as those from which the greatest amounts of the active principle, was released in the colorimetric drug delivery assay, and from which the largest inhibition zone sizes were demonstrated by the medicament. Next to HO and HS were neat sheabutter (NS) and emulsifying ointment (EO) of approximately equal ranking as regards antimicrobial activities, while lanolin hydrous (LH) and lanolin anhydrous (LA) were the least-performing vehicles.

While salicylic acid at 1, 2, and 3% w/w concentrations in LH-based ointments was released to significantly greater measure than benzoic acid, salicylic acid yet exhibited poorer antibacterial and antifungal activities when compared to benzoic acid ointment products in the same vehicle. This vehicle (LH) was thus atypical with respect to the correlation of antimicrobial ointment activities with active-principle delivery propensity of vehicle. The antibacterial and antifungal activities of all the ointment product samples against the most sensitive organism species (*E. coli* and *C. pseudotropicalis*, respectively) were used for this analysis, which provided sufficient data for the comparison.

#### **Discussion:**

The efficacy of antimicrobial treatment of topical infections depends upon adequate release of the active principle(s) from ointment medication following application to skin or mucous membrane. Physical properties of the ointment vehicle largely determine efficiency of release of the active principle. This study has examined five hydrophilic ointment bases selected from among vehicles commonly used in research or preparation of medicated ointments (25,26,27) as possible alternatives to emulsifying ointment (EO), for improved delivery of benzoic acid and salicylic acid in topical antimicrobial medication. Improvement of an existing formulation may well involve the selection of a superior vehicle on grounds of its better intrinsic qualities for required therapeutic application of the active principle (28).

*In vitro* release studies are useful and used for prospective drug delivery efficacy testing, to evaluate the relative performance of comparable formulations and elucidate interactions between the active substance(s) and the semisolid base in dermatological formulations development (29). The agar diffusion testing procedure is reputed to correlate significantly with absorption rate through the skin for solid medicaments dispersed in solid matrices (30,31). The rates and extents of release of benzoic acid and salicylic acid from the investigated bases, determined in the study, integrated the medicaments' delivery profiles from the respective vehicles and from the agar medium.

Active principle of small particle size ( $\leq 180\mu\text{m}$ ) was used in the study to aid its dissolution in the ointment matrix. However, the medicaments did not completely dissolve but were suspended (dispersed) in the base because their concentrations used (6% w/w benzoic acid and 3% w/w salicylic acid; same as for the official Whitfield ointment) far exceeded their respective aqueous solubility, to engender dissolution in the aqua-porous phase of hydrophilic bases' matrix so that particulate dispersion ointment products resulted. Undissolved drug in a dispersion ointment serves as the reservoir from which more drug is dissolved as the minute amount in solution is liberated. Drug release from suspension-type ointments is reportedly influenced by its solubility, diffusion coefficient and concentration in the vehicle (21,32). The latter factor was corroborated in this study. Considering this factor, an alternative approach to enhancing release and efficacy of the active principles could be by increasing their concentration or modifying their ratio used in the ointments formula (33). However, the highest concentration of the active principle tested in this study was fixed at 6% because salicylic acid in topical use is known to be destructive to dermal tissue above that value (34).

#### **Appropriate physical consistency supports ointment utility**

Physical stability is a requirement for topical semisolid medicinal products. The commonly used dermal antimicrobial ointments (e.g. of benzoic acid) are required to be soft and unctuous but stiff enough to remain in place when applied while protective ointments (e.g. zinc oxide paste) should be hard and stiff remaining in place when applied to abraded skin surfaces, and ophthalmic ointments are required to be the softest type (35,36). The shear-thinning property of all bases used in the study was desirable, predicting ease of skin-surface spreading when the ointment would be

applied (37), and thus supporting its therapeutic function as well as ensuring storage stability of the product (38).

Consistency durability of semisolid medications (creams and ointments) is however affected by heat, hence ointments are required to be always stored in a cool place. But on application to skin, warm human body temperature raises that of the applied ointment also to 37°C. Therefore, temperature of 37°C is the appropriate and desirable softening or melting point for ideal ointments (37), so that the body temperature would enhance mobility of the incorporated medicament in the ointment vehicle and its release. All medicated ointments in this study showed softening point of 37°C or lower (Table 3) thus they met the suitability requirement. Also, the proximity of the softening and congealing points of each ointment base, being within  $\pm 1^\circ\text{C}$  (except for HS only) revealed that the internal three-dimensional network structure of the base, formed by its components (38), was much stable, withstanding disruptive effect of heat stresses applied.

On the other hand, the relatively higher vulnerability of HS base (made from NS by fusion) to heat stress was clearly attributable to its polymorphic character shown also by the significant drop in viscosity of its ointment products, accompanied with water-leaching. Sheabutter is known to exhibit polymorphism, transforming under thermal stress into weaker-texture (unstable) form having lower softening and melting point values than its stable form prior to heating (39). The physical consistency weakness of HS base evident in this study was, however, advantageous for drug release, in that it permitted more extensive delivery and greater antimicrobial activities of the incorporated medicaments than most other bases.

#### **Factors contributing to drug delivery in ointment**

Concentration of the active principle and appropriate selection of the ointment vehicle exert a prominent influence on the amounts and activities of the medicament released from ointment products, as this study has further substantiated. Benzoic acid and salicylic acid ointment formulated in four bases (HO, HS, NS, and EO) thus demonstrated drug release and antimicrobial activities superior to ointments made with other bases (LA or LH). Ignoring data of the latter two bases, trend-agreement was evident comparing the *in vitro* drug release data with the antimicrobial activity results. Other factors showing potential influence on delivery of antimicrobial medicaments in ointments studied were physical and formulation factors (heat stress and medicament intro-

duced into vehicle, respectively), which altered ointment base consistency and stability in HS and NS-based products giving weaker-texture and lower-viscosity outcomes. The processing methods (fusion and levigation) were other factors, which caused polymorphism-related "bleeding" of the HS-based ointments. The HS bleeding was a reversal of water-incorporation into the base achieved at its compounding stage, and a form of syneresis. Syneresis is the separation of liquid (often the solvent phase) from polymeric materials (e.g. gels) constituted by aqueous and non-aqueous macromolecular units; a form of instability caused by shrinking of the three-dimensional gel network holding the liquid phase within the stable polymer structure (36).

Consistency alteration of ointment should expectedly influence its drug delivery rate particularly on ageing, because the rheological property determines the value of diffusion coefficient of the active substance in the formulation (1,29). Incorporation of the medicament into ointment base in this study generally caused decrease, but sometimes increase, of viscosity of the resulting medicated ointments. Sinko (40) had stated that the rheological property of semisolid products is altered by incorporation of medicament into the vehicle, spreading the product on skin, or by milling operation. Yet, statistical comparison of the physical consistency parameter (viscosity values) of different ointment preparations of this study to release rates of their incorporated medicament was found to be not significant. Lack of significant impact of the rheological property of pharmaceutical semisolids on drug release has been similarly noted in earlier studies (41,42).

#### **Conclusion:**

Benzoic acid exhibited stronger antimicrobial activities against most test organisms than salicylic acid in ointment preparations. There was correlation between release and antimicrobial activities of the medicaments in ointments produced using bases that permitted ample delivery of incorporated medicament. The drug delivery and activity appraisals increased with increase of drug concentration. Hydrous ointment and hydrous sheabutter bases demonstrated the highest levels of both antibacterial and antifungal properties, and the greatest amounts of the active principles released; followed by neat sheabutter and emulsifying ointment. Water number of the bases, or viscosity or heat-stress tolerance of bland or medicated ointments and method of ointment preparation did not demonstrate any direct influence on the rate of drug delivery from ointment vehicles.



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## Original Article

## Open Access

## Hygiene quality of traditional and industrial table olives from markets in Rabat-Salé and Temara cities in Morocco

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### Abstract:

**Background:** Table olives are one of the most important vegetable canning products in Morocco, which is considered one of the world's largest producing countries. Currently, many outlets prepare table olives by different methods that do not comply with standard hygiene practices. Hence, this research was conducted to assess the quality standard of these olives by evaluating their physico-chemical and microbiological properties.

**Methodology:** A total of 108 samples of table olives (pitted green olives and blacks) obtained from Rabat-Salé and Rabat-Temara markets in Morocco were evaluated. Physico-chemical properties of the olives including pH, oxido-reduction potential (ORP) and titrable acidity were determined using the analytical methods of the Association of Official Analytical Chemists (AOAC). Microbiological analyses including standard plate count (SPC) for total aerobic mesophilic flora (TAMB), total coliforms (TC), faecal coliforms (FC), yeasts, clostridia, *Staphylococcus aureus*, faecal streptococci and salmonella counts, were performed using standard microbiological methods. The identification of yeast isolates was carried out with the commercial API 20C biochemical identification kit.

**Results:** The average microbial loads for traditional olive samples were  $3.2 \times 10^6$  CFU/ml for SPC,  $1.7 \times 10^4$  CFU/ml for TC,  $8.7 \times 10^3$  CFU/ml for FC, and  $2.5 \times 10^6$  CFU/ml for yeast, which were higher compared to the average microbial loads of industrial olives with values of  $5.9 \times 10^5$  CFU/ml,  $5 \times 10^1$  CFU/ml, 0 CFU/ml and 0 CFU/ml respectively. One hundred percent (56 of 56) of the traditional olives (pitted green and black) from Temara-Rabat markets were contaminated with coliforms while 50% of green and 65% of black olives in Salé-Rabat were contaminated with coliforms. Five percent (5%) each of the traditional green and black olives in Salé-Rabat markets were contaminated with clostridia (spore forming bacteria). No FC or other bacteria and yeasts were present in the industrial olives, and none of the olives was contaminated with *S. aureus*, faecal streptococci and salmonella. Of the total of 8 yeast strains isolated from the traditional olives, 4 (50%) were *Candida guilliermondii*, 2 (25%) *Candida lusitanae* and 2 (25%) *Candida famata*.

**Conclusion:** The contamination of olive oil products may be due to different sources such as water, processing materials, storage condition, cleaning, labour and others. There is need for increase awareness and control of these at the points of sale of these traditional olives.

**Keywords:** hygiene; physico-chemical properties; microbiology; traditional olives; quality

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## Qualité Hygiène des olives de table traditionnelles et industrielles des marchés des villes de Rabat-Salé et Témara au Maroc

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### Resume:

**Contexte:** Les olives de table sont l'un des produits de mise en conserve de légumes les plus importants au Maroc, qui est considéré comme l'un des plus grands pays producteurs du monde. Actuellement, de nombreux

points de vente préparent les olives de table par différentes méthodes non conformes aux pratiques d'hygiène standard. Ainsi, cette recherche a été menée pour évaluer le standard de qualité de ces olives en évaluant leurs propriétés physico-chimiques et microbiologiques.

**Méthodologie:** Un total de 108 échantillons d'olives de table (olives vertes dénoyautées et noires) obtenus sur les marchés de Rabat-Salé et Rabat-Témara au Maroc ont été évalués. Les propriétés physico-chimiques des olives, y compris le pH, le potentiel d'oxydoréduction (ORP) et l'acidité titrable ont été déterminées en utilisant les méthodes analytiques de l'Association of Official Analytical Chemists (AOAC). Les analyses microbiologiques, y compris la numération sur plaque standard (SPC) pour la flore mésophile aérobie totale (FMAT), les coliformes totaux (CT), les coliformes fécaux (CF), les levures, les clostridies, *Staphylococcus aureus*, les streptocoques fécaux et les numérations de salmonelles, ont été effectuées à l'aide de méthodes microbiologiques standard. L'identification des isolats de levure a été réalisée avec le kit d'identification biochimique API 20E du commerce.

**Résultats:** Les charges microbiennes moyennes pour les échantillons d'olives traditionnelles étaient de  $3,2 \times 10^6$  UFC/ml pour le SPC,  $1,7 \times 10^4$  UFC/ml pour le TC,  $8,7 \times 10^3$  UFC/ml pour le FC et  $2,5 \times 10^6$  UFC/ml pour la levure, qui étaient plus élevées par rapport aux charges microbiennes moyennes des olives industrielles avec des valeurs respectives de  $5,9 \times 10^5$  UFC/ml,  $5 \times 10^1$  UFC/ml, 0 UFC/ml et 0 UFC/ml. Cent pour cent (56 sur 56) des olives traditionnelles (dénoyautées vertes et noires) des marchés de Témara-Rabat étaient contaminées par des coliformes tandis que 50% des olives vertes et 65% des olives noires de Salé-Rabat étaient contaminées par des coliformes. Cinq pour cent (5%) de chacune des olives vertes et noires traditionnelles des marchés de Salé-Rabat étaient contaminées par des clostridia (bactéries sporulantes). Aucune FC ni aucune autre bactérie et levure n'étaient présentes dans les olives industrielles, et aucune des olives n'était contaminée par *S. aureus*, des streptocoques fécaux et des salmonelles. Sur un total de 8 souches de levure isolées des olives traditionnelles, 4 (50%) étaient *Candida guilliermondii*, 2 (25%) *Candida lusitanae* et 2 (25%) *Candida famata*.

**Conclusion:** La contamination des produits à base d'huile d'olive peut être due à différentes sources telles que l'eau, les matériaux de traitement, les conditions de stockage, le nettoyage, la main-d'œuvre et autres. Il est nécessaire d'accroître la sensibilisation et le contrôle de ceux-ci dans les points de vente de ces olives traditionnelles.

**Mots-clés:** hygiène; propriétés physico-chimiques; microbiologie; olives traditionnelles; qualité

## Introduction:

Olive tree is a specific tree of the Mediterranean basin whose likely origin is Egypt, India, Syria or Ethiopia. Its cultivation in North Africa existed before the arrival of the Romans and the production of its oil was recognized about 7,000 years ago (1). In Morocco, the national production of table olives is about 2 million tons in year (2) for an area of 957,000 hectare, and the most widely available variety is the Moroccan picholine (Zitoune beldi). The regions in Morocco with highest production of olives are Marrakech, Safi, Beni Mellal, Khénifra, Tangier-Tétouan and Fés-Méknés. The three methods of olive preparations mostly practiced in Morocco are; the Spanish method for green olives, the Californian method for oxidized black olives and the Greek method for black olives, but the method most often used by industrialists is the Spanish preparation method which is an alkaline desamerization treatment of olives.

The presence of staphylococci, faecal streptococci, total coliforms, yeasts and molds contaminating table olives have been reported by many researchers in Morocco, especially in the regions of Rabat and Marrakech (3,4). In addition, the presence of penicillium spores in olive samples (especially black olives) was reported by Maouni et al., (5) and Lamrani et al., (6) in the region of Fez Marrakech. Outside of Morocco, Caggia et al., (7) has isolated and identified *Listeria monocytogenes* in olive samples of traders in Italy, and fecal coliforms, streptococci and reductive sulfite clostridia were isolated from samples of commercial olives in Portugal (8). The objective of this

study is to assess hygiene quality and identify the most predominant yeast species in traditional and industrial table olives sold in the markets of two cities in Morocco.

## Materials and method:

### Sampling:

A total of 108 pitted black and green olive samples (96 traditional and 12 industrial) from markets in Temara-Rabat and Salé-Rabat in Morocco were collected for evaluation of the hygienic quality of these products for direct consumption. For each of the traditional black and green olive brands, 4 samples were collected from 7 points of sales from markets in both Temara-Rabat and Salé-Rabat. For the industrial olives, 6 samples were collected for each black and green olives (3 per brand).

Samples were delivered to the laboratory directly in a cooler. The maximum time between sampling and sample analysis was one hour. All samples were analysed by and results obtained compared with national and international standards.

### Physico-chemical analysis of olive samples

The pH and oxido-reduction potential (ORP) of samples were measured from a 20% dry matter solution using a multi-parameter measurement pH after the device has been calibrated using AOAC method 981.12 (9). The liquid solution of the product was prepared and analyzed by titrimetry at pH 8.1 with 0.1N sodium hydroxide solution (NaOH) using AOAC methods 920.149 (c), 942.15A and 942.15B (9). Total acidity of olives was expressed by convention in grams of citric acid.

### Standard plate count (SPC) for total aerobic mesophilic flora

The standard plate count (SPC) for total aerobic mesophilic flora (TAMF) was done after appropriate sample dilutions in peptone water buffered broth and subsequent seeding on the plate count agar (PCA) growth medium and incubation at 30°C for 72 hours (10).

### Total and fecal coliform counts

Total coliform (TC) and faecal coliform (FC) counts were done by culturing appropriate sample dilution of olives on MacConkey agar plate and incubating at 30°C for TC and 44°C for FC. After 24 hours of incubation, red colonies were counted (11).

### *Staphylococcus aureus* (SA) count:

*Staphylococcus aureus* count was performed by inoculating Baird Parker culture medium with appropriate olive sample dilution and incubating aerobically at 37°C for 24 hours (12).

### Faecal streptococci (FS) count

Faecal streptococci count was done on Rothe broth and after incubation at 37°C for 24 hours, positive tubes were seeded on Litsky broth and incubated at 37°C for 24 hours (13).

### Salmonella count

Pre-enrichment was done by adding 25ml of olive sample to 225ml of sterile peptone water dabbled in a 250ml Erlenmeyer flask, which was incubated at 37°C for 12 hrs. Enrichment was done using two broths; Muller Kaufman and tetrathionate (MKTn) broth (Merck, Germany). MKTn tubes showing positive result were sub-cultured onto XLDA agar for *Salmonella*, where positive colonies appeared green (14). Identification was done by the procedure described by Poelma (15).

### Reducing sulfite count for anaerobic spore forming bacteria (SFB)

The count for anaerobic spore forming bacteria (clostridia) was performed on Sodium Sulphite - Polymyxin - Cysteine Sulphite (SPS) medium. The sample solution was first heat-treated at 80°C for 10 minutes, after which SPS medium was seeded and incubated at 30°C for 24-48 hours. Only black colonies were counted (16).

### Lactic acid bacteria count

Lactic acid bacteria count was carried

out using Man Rogosa and Sharpe (MRS) medium. Incubation was done at 30°C for mesophilic species and 45°C for thermophilic species for 48 hours. Round shape or lenticular colonies were counted (17).

### Yeast enumeration and identification

The method used consists of seeding Potato Dextrose Agar (PDA) that has been highly acidified (pH 3-3.5) by lactic acid. The count was carried out after 3 days of incubation at 37°C for yeasts and after 4 days of incubation at 30°C for moulds (18). The identification of yeast isolates was carried out using the commercial biochemical API 20E kit (19).

## Results:

The physico-chemical analysis of the traditional green and black olive samples from the different outlets showed average pH, acidity and oxido-reduction potential (OPR) values for green olives of 4.4; 11.8 and 135.5 respectively, while for the black olives, the respective values were 6.3, 8.1 and 8.0. For the industrial olives, the values of pH, acidity and OPR of the black olives are respectively 4.5, 5.5 and 129.5 while the respective values for green olives are 5.9, 8.5 and 92.5 (Table 1).

Microbiological analyses of the black olive samples showed the average microbial loads for traditional olive samples as;  $3.2 \times 10^6$  for SPC,  $1.3 \times 10^4$  for TC,  $8.7 \times 10^3$  for FC, and  $2.5 \times 10^6$  for yeast, which were higher compared to the average microbial loads of industrial olives with values of  $5.9 \times 10^5$ ,  $5 \times 10^1$ , 0 CFU/ml and 0 CFU/ml respectively. One hundred percent (56 of 56) of the traditional olives (pitted green and black) from Temara-Rabat markets were contaminated with coliforms (TC and FC) while 50% of green and 65% of black olives in Salé-Rabat markets were contaminated with coliforms.

Five percent (5%) each of the traditional green and black olives in Salé-Rabat were contaminated with clostridia (spore forming bacteria). No FC or other bacteria and yeasts were present in the industrial olives, and none of the olives was contaminated with *S. aureus*, faecal streptococci and salmonella. Of the total of 8 yeast strains isolated from the traditional olives, 4 (50%) were *Candida guilliermondii*, 2 (25%) were *Candida lusitanae* and 2 (25%) were *Candida famata* (Fig 1).

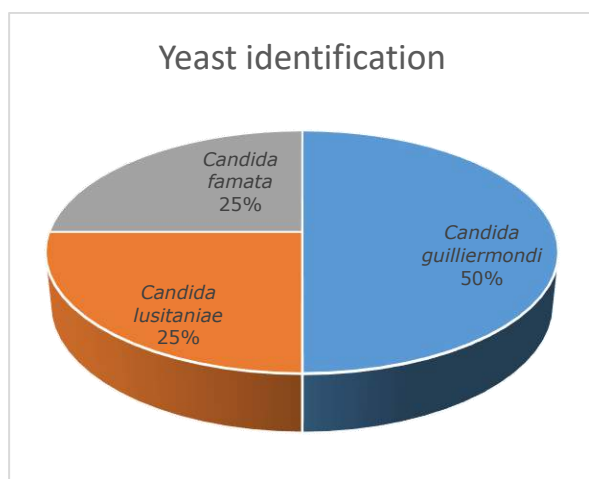
Table 1: Physico-chemical composition of traditional and industrial pitted olives in Morocco

Type of olives	Point of sale (PS)	Number of sample	pH			Acidity °D			Oxido-reduction potential ORP (mV)		
			Min	Max	Average	Min	Max	Average	Min	Max	Average
Traditional pitted green	PS1 to PS7 (Rabat-Temara)	28	3.34	4.62	4.17	7.7	19.7	11.8	111.6	200.8	144.6
	PS8 to PS12 (Rabat-Salé)	20	-	-	4.60	-	-	-	-	-	123.5
	Total	48			4.35			11.8			135.5
Traditional pitted black	PS1 to PS7 (Rabat-Temara)	28	6.08	7.69	6.90	7.50	9.00	8.07	-35.10	27.60	-4.11
	PS8 to PS12 (Rabat-Salé)	20	-	-	5.54	-	-	-	-	-	24.8
	Total	48			6.3			8.07			8
Industrial pitted green	Brand 1 and 2	6	5.43	6.39	5.91	3.00	14	8.5	17.70	74.83	92.53
Industrial pitted black	Brand 1 and 2	6	4.51	4.57	4.54	4.66	6.33	5.49	127.26	131.86	129.56

Min: Minimum; Max: Maximum

Table 2: Microbial contamination of traditional and industrial olives in Morocco

Olive type	Point of sale (PS)	Number of sample	SPC (10 <sup>5</sup> CFU/ml)			TC (10 <sup>2</sup> CFU/ml)			FC (10 <sup>2</sup> CFU/ml)			StaA	FStr	Salm	SFB	Lactic acid bacteria	Yeast
			Min	Max	Av	Min	Max	Av	Min	Max	Av	10 <sup>3</sup> CFU/ml	10 <sup>3</sup> CFU/ml	CFU/ml	10 <sup>3</sup> CFU/ml	10 <sup>5</sup> CFU/ml	10 <sup>6</sup> CFU/ml
Traditional green	PS1 to PS7 (Temara-Rabat)	28	8.7	51	32	5.5	327	130	5.2	228	87.5	0	0	0	0	2.53	2.5
	PS8 to PS12 (Salé-Rabat)	20				28 (100%) contaminated			10 (50%) contaminated			0	0	0	1 (5%) contaminated		>10
Traditional black	PS1 to PS7 (Temara-Rabat)	28	12	45	30	50	450	171	14	227	82	0	0	0	0	15.2	98
	PS8 to PS12 (Salé-Rabat)	20				28 (100%) contaminated			13 (65%) contaminated			0	0	0	1 (5%) contaminated		>10
Industrial green	Brand 1 and 2	6	2.4	9.4	5.9	0	1	0.5	0	0	00	0	0	0	0	0	0
Industrial Black	Brand 1 and 2	6	1	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0

TC: coliforms; FC: fecal coliforms; SPC: Standard Plate Count; FStr: fecal streptococci; SFB: spore forming bacteria; StaA: *Staphylococcus aureus*; Salm: salmonella; Min: minimum; Max: maximum; Av: average; CFU: colony forming unitFig 1: *Candida* species isolated from table olives in Morocco

## Discussion:

The oxido-reduction potential (ORP) of traditional green olives in this study was very high compared to that of the black olives of the same type, but almost similar to that of industrial green olives, therefore the pH of green olive is acidic compared to black olive

which is close to neutrality. The pH is correlated with the amount of free fatty and organic fatty acids produced by microorganisms (20), and the recommended maximum pH value should be less than 4.3 (21). However, the values obtained in our study are higher than those reported previously (22). The acidity values reported by the IOC (pH 0.3 - 0.5) and those by Kailis and Harris (23), and Ünal and Cevdet (24) were lower than our values, which may relate to the treatment conditions and lactic acid activity of the olives.

Traditional green and black olive samples from Témara-Rabat markets were more contaminated with coliforms (100%) when compared to those from Salé-Rabat markets with 50% and 65% contamination for green and black olives respectively, and 5% of both olive types were contaminated by clostridia. Coliforms and clostridia contaminations have been reported in traditional olives in Portugal (8) and in Marrakech region of Morocco (3). In another study, Maouni et al., (5) reported that household-prepared table olives had more microbial loads than the commercial olives. The high aerobic flora load in the traditional

green olive samples can be explained by the low salt content (less than 6%) which increase acidity, and by prolonged storage at the orchard level, which increase exposure to microbial contaminants. Also, there may be proliferation of microorganisms at the traditional olive preparation and extended open-air storage at room temperature. However, there was no such contamination with the industrial olives, probably the result of heat treatment and good hygiene during preparation, which therefore make them safe for consumption.

For the yeast contents, the values reported in this study exceed the recommended standards, which can lead to the deterioration of the olives with release of CO<sub>2</sub>, resulting in bad odors (25). *Candida guilliermondii* was the most commonly identified yeast specie in the contaminated olive samples. This species has been reported as essential for fermentation of traditional olives in Italy (26) and Morocco (27,28). *Candida famata* and *C. lusitaniae* were the other species identified in our study that have been reported as normal flora during fermentation process of olives in Turkey (29).

## Conclusion:

Microbiological analyses of the traditional olive samples show the presence of faecal flora especially clostridia in the samples, which is an indicator of poor hygienic conditions in the preparation of these olives. Our findings should inform preparers of the risks associated with poor hygiene in preparation of the olives, and encourage measures such as pasteurization, environmental and instrument cleanliness, availability of water sanitation and hygiene (WASH) facilities, proper packaging of finished products, and cooling, that can help reduce microbial contaminations during preparation.

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**Short Communication****Open Access****Evaluation of a cryptococcal antigen lateral flow assay test for rapid detection of cryptococcal infection in HIV-negative patients in Ibadan, Nigeria**<sup>\*1,2,4</sup>Fayemiwo, S. A., <sup>1,2</sup>Makanjuola, O. B., <sup>1</sup>Nwaokenye, J., and <sup>3</sup>Owolabi, M. O.<sup>1</sup>Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria<sup>2</sup>Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, Ibadan, Nigeria<sup>3</sup>Department of Medicine, College of Medicine, University of Ibadan, Ibadan, Nigeria<sup>4</sup>Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, University of Manchester, Manchester, M13 9PL, UK\*Correspondence to: [dayteet@yahoo.com](mailto:dayteet@yahoo.com)**Abstract:**

**Background:** A number of studies have been conducted in Nigeria on the prevalence of cryptococcal infections mostly on HIV-infected patients using culture, India ink and/or latex agglutination tests. These tests are either laborious, time-consuming and expensive or have low sensitivity, thus limiting their use. Cryptococcal antigen lateral flow assays (LFA) were introduced in the last decade as rapid user-friendly tests for diagnosis. In this study, we sought to determine the diagnostic accuracy of an LFA kit for the detection of cryptococcal antigen in the serum of HIV-negative patients with or without cerebrovascular accident (CVA) or stroke in University College Hospital, Ibadan, Nigeria.

**Methodology:** The diagnostic accuracy of Dynamiker CrAg LFA was tested against Biosynex<sup>R</sup> CryptoPS on serum samples of 100 HIV-negative patients with and without stroke. Samples were tested and results interpreted in accordance with the manufacturer's instructions. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios of the Dynamiker CrAg LFA were calculated by comparing with the Biosynex<sup>R</sup> CryptoPS as 'gold standard'.

**Results:** Overall, a total of 98 valid patient sample results were analysed; 17 samples (17.3%) were positive with Dynamiker CrAg LFA cryptococcal antigen and 16 samples (16.3%) were positive with Biosynex<sup>R</sup> CryptoPS. The sensitivity, specificity, PPV and NPV of Dynamiker CrAg LFA compared to the Biosynex<sup>R</sup> CryptoPS were 100%, 98.8%, 94.1% and 100% respectively, while the positive and negative likelihood ratios were 82 and 0 respectively.

**Conclusion:** In comparison to the Biosynex<sup>R</sup> CryptoPS, the Dynamiker CrAg LFA is a highly sensitive and specific test for the detection of cryptococcal antigen in serum. The test kit should be considered as a screening device for cryptococcal infection both in outreach and clinical settings, especially in antiretroviral therapy (ART) centres.

**Keywords:** Cryptococcus; evaluation; lateral flow assay; HIV-negative; stroke

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**Évaluation d'un test de test d'écoulement latéral de l'antigène cryptococcique pour la détection rapide d'une infection cryptococcique chez des patients séronégatifs à Ibadan, Nigéria**<sup>\*1,2,4</sup>Fayemiwo, S. A., <sup>1,2</sup>Makanjuola, O. B., <sup>1</sup>Nwaokenye, J., et <sup>3</sup>Owolabi, M. O.<sup>1</sup>Département de microbiologie médicale et de parasitologie, University College Hospital, Ibadan, Nigéria<sup>2</sup>Département de microbiologie médicale et de parasitologie, Collège de médecine, Université d'Ibadan, Ibadan, Nigéria<sup>3</sup>Département de médecine, Collège de médecine, Université d'Ibadan, Ibadan, Nigéria

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## Abstrait:

**Contexte:** Un certain nombre d'études ont été menées au Nigéria sur la prévalence des infections à cryptocoques principalement chez les patients infectés par le VIH en utilisant des tests d'agglutination en culture, à l'encre de Chine et / ou au latex. Ces tests sont soit laborieux, longs et coûteux ou ont une faible sensibilité, limitant ainsi leur utilisation. Les tests d'écoulement latéral de l'antigène cryptococcique (LFA) ont été introduits au cours de la dernière décennie en tant que tests conviviaux rapides pour le diagnostic. Dans cette étude, nous avons cherché à déterminer la précision diagnostique d'un kit LFA pour la détection de l'antigène cryptococcique dans le sérum de patients séronégatifs avec ou sans accident vasculaire cérébral (AVC) ou accident vasculaire cérébral à University College Hospital, Ibadan, Nigeria.

**Méthodologie:** La précision diagnostique de Dynamiker CrAg LFA a été testée contre Biosynex<sup>R</sup> CryptoPS sur des échantillons de sérum de 100 patients séronégatifs avec et sans accident vasculaire cérébral. Les échantillons ont été testés et les résultats interprétés conformément aux instructions du fabricant. La sensibilité, la spécificité, la valeur prédictive positive (PPV), la valeur prédictive négative (NPV) et les rapports de vraisemblance positifs et négatifs du Dynamiker CrAg LFA ont été calculés en les comparant avec le Biosynex<sup>R</sup> CryptoPS comme 'gold standard'.

**Résultats:** Au total, 98 résultats d'échantillons de patients valides ont été analysés; 17 échantillons (17,3%) étaient positifs avec l'antigène cryptococcique Dynamiker CrAg LFA et 16 échantillons (16,3%) étaient positifs avec Biosynex<sup>R</sup> CryptoPS. La sensibilité, la spécificité, le PPV et le NPV de Dynamiker LFA par rapport au Biosynex<sup>R</sup> CryptoPS étaient respectivement de 100%, 98,8%, 94,1% et 100%, tandis que les rapports de vraisemblance positifs et négatifs étaient respectivement de 82 et 0.

**Conclusion:** Par rapport au Biosynex<sup>R</sup> CryptoPS, le Dynamiker CrAg LFA est un test hautement sensible et spécifique pour la détection de l'antigène cryptococcique dans le sérum. Le kit de test doit être considéré comme un dispositif de dépistage de l'infection cryptococcique à la fois dans les milieux de proximité et cliniques, en particulier dans les centres de thérapie antirétrovirale (ART).

**Mots-clés:** Cryptococcus; évaluation; essai d'écoulement latéral; Séronégatif; accident vasculaire cérébral

## Introduction:

Cryptococcosis is a systemic fungal infection caused by *Cryptococcus neoformans* and *Cryptococcus gatti* complex (1). These fungi are considered potential pathogenic species and infect both human and animal hosts. While it is indeed true that *C. neoformans* infections are commoner among the immunocompromised, *C. gatti*, on the other hand is more frequently implicated in fungal infections among immunocompetent hosts. The commonest infections they cause in humans include meningitis, pneumonia and disseminated cryptococcosis, mostly in immunosuppressed hosts. Sporadic cases of these infections are reported all over the world. However, in recent times, the AIDS pandemic has been the significant factor for increased incidence of this disease. Cryptococcal meningitis is one of the common causes of unconsciousness and adult meningitis in Africa, (2) resulting in about 20-25% of AIDS-related deaths (3,4).

Although, the major risk factors for cerebrovascular accident (CVA) or stroke in the Nigerian population include hypertension, alcohol consumption, sedentary lifestyle, and impaired glucose tolerance, these factors do not entirely explain the occurrence of stroke. Meanwhile, patients with uncontrolled hyper-

tension who usually suffer stroke present at the hospital in coma in the Nigerian setting. Coma, being a common presentation of both cryptococcal meningitis and stroke, may thus present a diagnostic challenge, necessitating a rapid screening to exclude cryptococcal infection (5).

Laboratory diagnosis of cryptococcal infections have traditionally been carried out by CSF culture for isolation of the pathogen, India ink test and/or cryptococcal antigen latex agglutination tests. However, these tests are either laborious, time-consuming, expensive or have low sensitivity, thus limiting their clinical use. For instance, the 'gold standard' test for the diagnosis of cryptococcal meningitis is CSF culture but diagnosis by culture can take up to 1 or 2 weeks for definitive results. India ink test, though quick, has a low sensitivity resulting in missed infections (6). Thus, other test methods have been devised to expedite diagnosis and treatment, thus improving clinical outcome.

The cryptococcal antigen lateral flow assay (LFA) tests were introduced in the last decade of this century as rapid user-friendly tests for diagnosis of *Cryptococcus* infections. The use of these assays has simplified the presumptive diagnosis of cryptococcal infection as point-of-care test using serum/plasma, whole blood and cerebrospinal fluid (CSF). The LFA have been reported to demonstrate high accuracy

in these body fluids for the diagnosis of cryptococcosis in patients at risk of infection (7).

The first of these kits was manufactured by IMMY and has been validated and licensed for use in many countries. Other LFA kits such as the Biosynex<sup>R</sup> CryptoPS have been found to also perform well (6,8,9), with comparable diagnostic accuracy to the IMMY CrAg kit that is considered the 'gold standard' LFA test on serum samples (8). Furthermore, other newer test kits have been manufactured and are in need of evaluation. We therefore evaluated the diagnostic accuracy of the Dynamiker CrAg LFA test against Biosynex<sup>R</sup> CryptoPS using sera of HIV-negative patients with and without stroke in Ibadan, southwest Nigeria.

## Materials and method:

### Study setting

The study was conducted in Ibadan, southwestern Nigeria as part of the Stroke Investigative Research and Education Network (SIREN) project, a multicentre and multidisciplinary study on stroke in sub-Saharan Africa.

### Study design, subjects and sample collection

This was a comparative evaluation study of the diagnostic accuracy of the cryptococcal CrAg LFA (Dynamiker Biotechnology, Tianjin Co., Ltd,) against Biosynex<sup>R</sup> CryptoPS kits on sera of 100 HIV-negative adult patients aged  $\geq 18$  years with and without stroke, randomly selected from the SIREN project.

Approximately 5ml of venous blood samples were collected from each patient. Blood samples were allowed to clot and serum was separated by centrifugation and stored at  $-80^{\circ}\text{C}$  until use. The study was approved by the University of Ibadan/University College Hospital Ethics Review Committee.

### Sample testing by the lateral flow assay kits

The serum samples were allowed to thaw for at least one hour at room temperature, and then tested against the Dynamiker CrAg (LOT 17040) and Biosynex<sup>R</sup> CryptoPS kits strictly in accordance with the manufacturer's instructions.

### Principle of the lateral flow device

Both test kits use the lateral flow technique and double antibody sandwich format. In the kit, the test cassette consists of *Cryptococcus* antibodies bound to gold particles and coated on fibre glass. The cassette has a test line (T) and a control line (C) on nitrocellulose membrane which are coated with *Cryptococcus* and goat anti-mouse antibodies

respectively. The cryptococcal antigens if present in the test samples form antigen-antibody complexes with the gold-conjugated antibodies and migrate further along the nitrocellulose membrane. The antibodies immobilized on the test line (T) interact with the above complexes causing a visible red line. The wicking of samples causes free gold-conjugated antibodies to reach the control line (C) to form red colour. The absence of cryptococcal antigens in negative samples only cause a colour change in the control line (C), which is essentially a quality control measure to ensure the test is visually validated to be functioning properly.

### Procedural steps

Following strictly the instructions of the manufacturer for the Dynamiker CrAg LFA, the test kit was removed from the pouch and placed on a flat surface. 80 $\mu\text{L}$  of the serum sample was gently dispensed onto the sample pad and result read within 15-20 minutes, and interpreted as positive, negative or invalid. Where interpretation was difficult due to high viscosity of serum sample, such sample was re-centrifuged and the test repeated.

For the Biosynex<sup>R</sup> CryptoPS, the test kit was similarly removed from the pouch and placed on a flat, horizontal surface. 20 $\mu\text{L}$  of the serum sample was dispensed into the well, followed by 3 drops of the diluent. Result was read and interpreted as positive, negative or invalid in accordance with the manufacturer's instructions.

### Data analysis

The sensitivity, specificity, positive and negative predictive values of the Dynamiker CrAg test were calculated in reference to the Biosynex<sup>R</sup> CryptoPS using the formula; sensitivity =  $\text{TP}/(\text{TP} + \text{FN}) \times (100)$ , specificity =  $\text{TN}/(\text{TN} + \text{FP}) \times (100)$ , PPV =  $\text{TP}/(\text{TP} + \text{FP}) \times (100)$  and NPV =  $\text{TN}/(\text{TN} + \text{FN}) \times (100)$ , where TP=True Positive, TN=True Negative, FP=False Positive and FN=False Negative. The positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were also computed as follows;  $\text{NLR} = (1 - \text{sensitivity})/\text{specificity}$  and  $\text{PLR} = \text{sensitivity}/(1 - \text{specificity})$ .

## Results:

A total of 100 serum samples from 100 HIV-negative patients with or without stroke were tested by both the Dynamiker CrAg LFA and Biosynex<sup>R</sup> CryptoPS kits. After the initial test, 12 samples produced invalid results for the two kits but on repeat testing, only 2 samples remained invalid. A total of 98 sample results were therefore analysed. Seventeen samples

(17.3%) were positive for Dynamiker CrAg LFA while 16 (16.3%) were positive for Biosynex<sup>R</sup> CryptoPS assay (Table 1). The sensitivity, specificity, PPV and NPV of Dynamiker CrAg LFA compared to the 'gold standard' Biosynex<sup>R</sup> CryptoPS were 100%, 98.8%, 94.1% and 100% respectively. The positive and negative likelihood ratios were respectively 82 and 0 (Table 2).

Table 1: Cryptococcal antigen testing using Dynamiker CrAg LFA and Biosynex<sup>R</sup> CryptoPS assay kits

Diagnostic kit	Biosynex <sup>R</sup> CryptoPS		
	Positive	Negative	Total
<b>Dynamiker CrAg LFA</b>			
Positive	16	1	17
Negative	0	81	81
Total	16	82	98

sensitivity of Dynamiker CrAg LFA=16/16 (100%); Specificity=81/82 (98.8%); Negative Predictive Value (NPV)=81/81 (100%); Positive Predictive Value (PPV)=16/17 (94.1%); Negative Likelihood Ratio = 0.0; Positive Likelihood Ratio = 82.0; Disease Prevalence = 16.3%.

Table 2: Summary of the diagnostic performance of Dynamiker CrAg LFA compared to the 'gold standard'

Characteristics	Sensitivity	Specificity	NPV	PPV
Value (%)	100	98.8	100	94.1
95% CI	79.4–100	93.4–99.9	92.4–99.8	69.5–99.1

NPV=Negative Predictive Value; PPV=Positive Predictive Value; CI=Confidence Interval

## Discussion:

In this study, the Dynamiker CrAg LFA was highly sensitive and specific in detecting cryptococcal antigen in the serum of HIV-negative patients with and without stroke when compared to Biosynex<sup>R</sup> CryptoPS. The negative predictive value was 100% while the positive predictive value was 94.1%. These results imply that the probability of cryptococcal disease not being present when the test is negative is 100%, while the probability that the disease is present when the test is positive is 94.1%. The positive likelihood ratio was greater than 20 while the negative likelihood ratio was less than 0.1, which proved that ill people with cryptococcal infection are more likely to have abnormal test results compared to healthy people.

The Dynamiker CrAg test was simple to use and rapid in detecting cryptococcal antigen in the serum samples of the patients. The test requires minimal training or equipment and therefore should be considered as a screening device both in outreach and clinical setting especially in ART centres.

Our study was limited by the inability to perform this evaluation with the more preferred IMMY CrAg test that is considered to be the LFA 'gold standard' test for detecting cryptococcus infection. This limitation was however compensated for by the use of the Biosynex<sup>R</sup> CryptoPS kit which has been reported to have comparable diagnostic accuracy with IMMY CrAg test and is CE-marked for use in Europe (8).

## Conflict of interest:

Authors declare no competing interest

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Copyright AJCEM 2021: <https://dx.doi.org/10.4314/ajcem.v22i1.13>**Short Communication****Open Access****Positivity yield of HIV index testing services from selected healthcare facilities in Ondo State, southwest Nigeria**Emeh, A., \*Usman, S. O., Adebajo, A. M., Ogboghodo, E., Akinbinu, B., Suraju, A.,  
Udechukwu, C., Ale, J., Ariyo, A., Owolagba, F. E., Jolayemi, T., and Okonkwo, P.

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\*Correspondence to: [senatorhopsy@yahoo.com](mailto:senatorhopsy@yahoo.com)**Abstract:**

**Background:** Index testing is a voluntary process whereby HIV seropositive clients are counselled and, after obtaining consent, their sexual and needle sharing partners are offered HIV testing services. Index testing has been associated with high HIV positivity yield. The aim of this study is to determine the positivity yield and identify factors influencing the yield from index testing strategy in selected healthcare facilities in Ondo State, southwest Nigeria.

**Methodology:** Six public hospitals in Ondo State with the highest HIV clients currently on treatment were selected. Records of all clients newly diagnosed to be HIV positive at the selected facilities from June 2018 to September 2019, and who had an outcome for index testing services were reviewed. Data were collected using a chart abstraction template from the index testing registers. Information collected included age and gender of the index clients and their partners, method of referral and notification of partners, HIV test results of partners and linkage status of new HIV positive partners. Data analyses were done using the Statistical Package for the Social Sciences (SPSS) software version 24.0. Chi-square was used to test association between variables at a significance level of  $p < 0.01$ .

**Results:** The records of a total of 904 index clients and their partners were reviewed with partner elicitation ratio of 1:1. The mean ages of index clients and their partners were  $38.52 \pm 10.96$  and  $38.98 \pm 10.79$  years respectively, and majority of the index clients (34.6%) and partners (35.5%) were in the 35-44 years age group. A total of 548 index clients were females (60.6%) while 528 of their partners were males (58.4%), indicating predominantly heterosexual (96.4%) and few homosexual (lesbian) relationships (3.6%). One-fifth (20%) of partners tested positive for HIV and were all (100%) linked to antiretroviral therapy (ART). The HIV positivity rate in partners of male index clients (26.9%) was significantly higher than in partners of female index clients (15.5%) ( $p < 0.01$ ). Partner referral method was mostly through assisted referral (56%) and most (82%) were contacted by the index clients through phone.

**Conclusion:** Due to its high positivity yield, index testing is a veritable strategy to increase HIV case detection and linkage to ART. Hence, proper deployment of index testing will be critical to improving ART coverage and achieving epidemiological control.

**Keywords:** HIV, client; partner; index testing; ART; southwest Nigeria

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**Rendement positif des services de dépistage de l'indice du VIH dans certains établissements de santé de l'État d'Ondo, dans le sud-ouest du Nigéria**Emeh, A., \*Usman, S. O., Adebajo, A. M., Ogboghodo, E., Akinbinu, B., Suraju, A.,  
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\*Correspondance à: [senatorhopsy@yahoo.com](mailto:senatorhopsy@yahoo.com)**Abstrait:**

**Contexte:** Le test d'indexation est un processus volontaire par lequel les clients séropositifs pour le VIH sont

conseillés et, après avoir obtenu leur consentement, leurs partenaires sexuels et de partage de seringues se voient offrir des services de dépistage du VIH. Le test d'index a été associé à un rendement élevé de positivité au VIH. Le but de cette étude est de déterminer le rendement de positivité et d'identifier les facteurs influençant le rendement de la stratégie de test d'index dans certains établissements de santé de l'État d'Ondo, dans le sud-ouest du Nigéria.

**Méthodologie:** Six hôpitaux publics de l'État d'Ondo avec les clients VIH les plus élevés actuellement sous traitement ont été sélectionnés. Les dossiers de tous les clients nouvellement diagnostiqués séropositifs dans les établissements sélectionnés de juin 2018 à septembre 2019 et qui ont obtenu un résultat pour les services de test d'index ont été examinés. Les données ont été collectées à l'aide d'un modèle d'abstraction de graphique à partir des registres de test d'index. Les informations recueillies incluaient l'âge et le sexe des clients de l'indice et de leurs partenaires, la méthode d'orientation et de notification des partenaires, les résultats des tests de dépistage du VIH des partenaires et le statut de liaison des nouveaux partenaires séropositifs. Les analyses de données ont été effectuées à l'aide du logiciel Statistical Package for the Social Sciences (SPSS) version 24.0. Le chi carré a été utilisé pour tester l'association entre les variables à un niveau de signification de  $p < 0,01$ .

**Résultats:** Les dossiers d'un total de 904 clients index et de leurs partenaires ont été examinés avec un ratio de sollicitation des partenaires de 1:1. L'âge moyen des clients de l'indice et de leurs partenaires était de  $38,52 \pm 10,96$  et  $38,98 \pm 10,79$  ans respectivement, et la majorité des clients de l'indice (34,6%) et des partenaires (35,5%) appartenaient au groupe d'âge des 35-44 ans. Un total de 548 clients index étaient des femmes (60,6%) tandis que 528 de leurs partenaires étaient des hommes (58,4%), indiquant principalement des relations hétérosexuelles (96,4%) et peu de relations homosexuelles (lesbiennes) (3,6%). Un cinquième (20%) des partenaires étaient positifs pour le VIH et étaient tous (100%) liés à un traitement antirétroviral (ART). Le taux de positivité pour le VIH chez les partenaires des clients de sexe masculin (26,9%) était significativement plus élevé que chez les partenaires des femmes de sexe féminin (15,5%) ( $p < 0,01$ ). La méthode de référence des partenaires se faisait principalement par référence assistée (56%) et la plupart (82%) étaient contactés par téléphone par les clients indexés.

**Conclusion:** En raison de son rendement élevé de positivité, le test d'indexation est une véritable stratégie pour augmenter la détection des cas de VIH et le lien avec le TAR. Par conséquent, un déploiement approprié des tests d'indexation sera essentiel pour améliorer la couverture des TAR et réaliser le contrôle épidémiologique.

**Mots-clés:** VIH, client; partenaire; tests d'index; ART; sud-ouest du Nigeria

## Introduction:

Index testing (IT) is a voluntary process where trained health workers, and lay providers, ask people diagnosed with HIV about their sexual partners or drug injecting partners and, with the consent of the HIV-positive client, offer these partners voluntary HIV testing (1). The sexual partners and drug injecting partners of people diagnosed with HIV infection have an increased probability of also being HIV-positive. Index testing services does not always require disclosure to the partner and may be anonymously conducted. These services are an efficient and effective way to diagnose people with HIV, link persons to HIV care, and identify partners in need of HIV prevention services (1). Index testing has been an important public health approach in infectious disease case detection and control for decades. This strategy has also been a part of programs for sexually transmitted infections and tuberculosis but has not previously been routinely implemented for HIV program (2).

Index testing increase uptake of HIV testing services among partners of people with HIV, result in high positivity yield, and increased linkage to treatment and care among partners of people with HIV. Other benefits of index testing include mutual support to access HIV prevention, treatment and care services, improved adherence and retention on treatment, increased support for the prevention of mother-to-child transmission and prioritization of effect-

tive HIV prevention for serodiscordant couples such as condom use, antiretroviral therapy, and pre-exposure prophylaxis for HIV-negative partners (2). This innovative strategy increases access to HIV testing services for populations currently underserved including adolescent girls and young women, adolescent boys and young men, partners of women tested in antenatal clinics and key populations amongst others.

Index testing ensures that the partners of HIV positive clients benefit from opportunities to learn their HIV status and commence ART if tested HIV positive. Therefore, index testing has a role to play in achieving optimum ART coverage and epidemiological control. Partner referral methods could either be passive referral, where index clients are encouraged to disclose their status and suggest HIV testing to their partner(s) on their own; contract referral, where index clients enter into a contract with the provider to refer their partner(s) to HIV testing within an agreed time period, after that the provider contacts the partner(s) directly and offers HIV testing, while maintaining the anonymity of the index patient; provider referral, where providers directly contact partners of index patients to offer HIV testing; or dual referral where the provider accompanies the index patient when they disclose their status and offers HIV testing to their partner(s) (3).

In five observational studies, assisted partner notification was associated with incr-

eased uptake of HIV testing services (HTS) among identified partners compared to passive referral (4-7). The proportion of partners of index patients who tested HIV-positive ranged from 20 to 72% in both passive and assisted arms of the four trials. Among the observational studies, the highest proportion of partners testing HIV positive was 86%. In four studies that reported on couples, between 29 and 40% were in serodiscordant partnerships (8-11). In Tijuana Mexico, 46 HIV-positive men having sex with men (MSM) and transgender women (TW) were enrolled as index patients and 132 MSM/TW partners were elicited for index testing. Out of the notified partners, 39% tested for HIV and 28% of these were newly diagnosed as HIV-positive. Partners who were seen by the index patient more than once in the past 4 months and those who primarily had sex with the index patient in one of their homes were more likely to be notified for index testing (12).

The present study aims to provide further knowledge on the outcomes of index testing efforts in Nigeria. Its specific objectives are to determine the HIV positivity yield from index testing in selected healthcare facilities in Ondo State, southwest Nigeria, and to identify factors associated with higher positivity yield from index testing.

## Materials and method:

### Study setting

This study was conducted in six public healthcare facilities providing index testing services for the highest number of persons living with HIV (PLHIV) in Ondo State, southwest Nigeria from June 2018 to September 2019. The facilities were; State Specialist Hospital Akure, State Specialist Hospital Ikare, State Specialist Hospital Ondo, State Specialist Hospital Okitipupa, Federal Medical Centre Owo, and General Hospital Ore.

### Study design, subjects and data collection

The study is a retrospective review of all newly diagnosed HIV positive clients with an outcome for index testing in the six selected facilities. HIV diagnosis was made following the national HIV serial testing algorithm with rapid test kits. Records of a total of 904 consecutive HIV-positive index clients (with their partners, n=904) who received index testing services during the period of study were reviewed. Data were retrieved from the index testing registers using a chart abstraction template which contained columns for all relevant information necessary for the study. Omitted information in the register was sought from other program registers and patient care cards.

Information collected included age and gender of the index clients and their partners, method of referral and notification of partners, HIV test results of partners and linkage status of new HIV positive partners. Partner referral methods were; (i) passive referral, where index clients were encouraged to disclose their status and suggest HIV testing to their partner(s) on their own; (ii) contract referral, where index clients entered into a contract with the provider to refer their partner(s) to HIV testing within an agreed time period, after which the provider contacts the partner(s) directly and offers HIV testing, while maintaining anonymity of the index patient; (iii) provider referral, where providers directly contact partners of index patients to offer HIV testing; and (iv) dual referral where the provider accompanies the index patient when they disclose their status and offers HIV testing to their partner(s) (3).

### Data entry and analysis

Collated data were reviewed and checked for completeness. Data entry and analysis were done using the Statistical Package for the Social Sciences (SPSS) software version 24.0. Chi-square was used to test association between different groups at a significance level of  $p < 0.01$ .

### Ethical considerations

Ethical approval to conduct the study was obtained from the review team of the APIN Public Health Initiative, Akure, Ondo State. Confidentiality was maintained by ensuring that names, hospital numbers and contact details of clients were not captured in the chart abstraction tool.

## Results:

The records of 904 index HIV clients and 904 partners recruited with partner elicitation ratio of 1:1, were reviewed for the study in the 6 selected hospitals in Ondo State, southwest Nigeria. The State Specialist Hospital Okitipupa had the highest number of clients participating in the study with 184 (20.4%) while Federal Medical Centre, Owo had the lowest number of 105 (11.6%) (Table 1).

Table 1: Frequency distribution of HIV-positive index clients from six selected hospitals in Ondo State, southwest Nigeria

Hospital	Index client	
	Frequency (n= 904)	Percent (%)
Federal Medical Centre, Owo	105	11.6
Ore General Hospital	164	18.1
State Specialist Hospital, Akure	154	17.0
State Specialist Hospital, Ikare	155	17.1
State Specialist Hospital, Okitipupa	184	20.4
State Specialist Hospital, Ondo	142	15.7



The mean age of the index clients was  $38.52 \pm 10.96$  years, while that of the partners was  $38.98 \pm 10.79$  years, and majority of the index clients (34.6%) and partners (35.5%) were in the 35-44 years age group (Table 2). Five hundred and forty-eight (548) of the index clients were females (60.6%), while 528 of their partners were males (58.4%) indicating predominantly heterosexual (96.4%,  $n=528$ ) and few homosexual (lesbian) relationships (3.6%,  $n=20$ ).

The HIV positivity rate among partners of index clients was 20% (181/904), and they were all initiated on ART (100% linkage). The HIV results of both male and female partners

were not significantly associated with age group ( $p > 0.05$ ) but the HIV positivity rate of 28.7% (108/376) in the female partners was significantly higher than 13.8% (73/528) in the male partners ( $p < 0.01$ ) (Table 3). Similarly, the HIV positivity rate of 26.9% (96/356) in partners of male clients (who were all females) was significantly higher than the rate of 15.5% (85/548) among partners of female index clients who were mostly but not all males ( $p < 0.01$ ) (Table 4). Most (56.2%) of the partners were notified using the assisted/provider referral method, and only one client had the dual referral method while none used the contract referral method.

Table 2: Age group and gender distribution of index HIV-positive index clients and partners from six selected hospitals, Ondo State, southwest Nigeria

Age group (years)	Index clients			Partners		
	Male	Female	Total (%)	Male	Female	Total (%)
<15	2	2	4 (0.4)	0	1	1 (0.1)
15-24	11	55	66 (7.3)	14	30	44 (4.9)
25-34	74	191	265 (29.4)	138	146	284 (31.4)
35-44	119	194	313 (34.6)	189	132	321 (35.5)
45-54	100	76	176 (19.5)	119	53	172 (19.0)
55-64	33	25	58 (6.4)	46	12	58 (6.4)
$\geq 65$	17	5	22 (2.4)	22	2	24 (2.7)
Total	356 (39.4)	548 (60.6)	904 (100)	528 (58.4)	376 (41.6)	904 (100)
Mean age (years)	$38.52 \pm 10.96$			$38.98 \pm 10.79$		

Table 3: HIV status of partners in relation to age group and gender in selected hospitals, Ondo State, Southwest Nigeria

Age group (years)	Partner					
	Male		Female		Total	
	No tested	No positive for HIV (%)	No tested	No positive for HIV (%)	No tested	No positive for HIV (%)
<15	0	0	1	1 (100)	1	1 (100)
15-24	14	3 (21.4)	30	13 (43.3)	44	16 (36.4)
25-34	138	14 (10.1)	146	37 (25.3)	284	51 (17.9)
35-44	189	26 (13.8)	132	36 (27.3)	321	62 (19.3)
45-54	119	22 (18.5)	53	14 (26.4)	172	36 (20.9)
55-64	46	5 (10.9)	12	6 (50.0)	58	11 (18.9)
>65	22	3 (13.6)	2	1 (50.0)	24	6 (25.0)
Total	528	*73 (13.8)	376	*108 (28.7)	904	181 (20)

\*HIV positivity rate in female partners was significantly higher than in male partners ( $p < 0.01$ ) but there was no significant difference in HIV positivity rate in relation to age group ( $p > 0.05$ )

Table 4: HIV status of partners of index clients in relation to age group in selected hospitals, Ondo State, Southwest Nigeria

Age group (years)	No of partners of male index client	No positive for HIV (%)	No of partners of female index client	No positive for HIV (%)	Total no of partners of index client	Total no positive for HIV (%)
<15	1	0	0	0	1	0
15-24	30	10 (33.3)	14	2 (14.3)	44	12 (27.3)
25-34	146	32 (21.9)	138	18 (13.0)	284	50 (17.6)
35-44	132	36 (27.3)	189	27 (14.3)	321	63 (19.6)
45-54	53	11 (20.8)	119	27 (22.7)	172	38 (22.1)
55-64	12	6 (50.0)	46	7 (15.2)	58	13 (22.4)
>65	2	1 (50.0)	22	4 (18.2)	24	5 (20.8)
Total	356	*96 (26.9)	548	*85 (15.5)	904	181 (20)

\*HIV positivity rate among partners of male index client was significantly higher than the positivity rate among partners of female index client ( $p < 0.01$ )

Over 80% of the partners were contacted via index client by phone, and only about 1% by the provider in person (Table 5). There were no significant association of partner HIV test results with partner notification methods ( $p=0.84$ ) or with partner contact method ( $p=0.77$ ).

Table 5: HIV status of partners in relation to partner referral and client contact methods in selected hospitals, Ondo State, southwest Nigeria

Partner referral method	HIV test result		Total	<i>p</i> value
	Negative	Positive		
Dual Referral	1	0	1	0.84
Passive/Client Referral	314	81	395	
Provider Referral	408	100	508	
<b>Total</b>	<b>723</b>	<b>181</b>	<b>904</b>	
<b>Partner contact method</b>				
Index client by phone	591	151	742	0.77
Index client in person	97	20	117	
Provider by phone	25	8	33	
Provider in person	10	2	12	
<b>Total</b>	<b>723</b>	<b>181</b>	<b>904</b>	

## Discussion:

This study examined the outcome of index testing (IT) services in Ondo State, southwestern Nigeria. Six public hospitals with the highest PLHIV clients on treatment were studied. The hospitals were from different locations covering the three senatorial districts of the state. The facilities from the southern part of the state had more participants with 20.4% from State Specialist Hospital Okitipupa and 18.1% from Ore General Hospital, compared with those from the more northern zones of the state: State Specialist Hospital Ikare (17.1%) and Owo (11.6%).

The mean age of the index clients was  $38.52 \pm 10.96$  years, and about 0.5% of them were < 15 years of age. Majority (34.6%) of the clients were in the 35-44 years group, with 75.4% being in 25- 49 years, and 16.9% above 50 years. This is similar to the findings in north central Nigeria where no client in the study was <15 years with majority of the clients between 25-49 years (69.5%), while 17.1% were above 50 years of age (13). These findings may be due to higher prevalence of HIV infection among the adult population, and challenges associated with access to IT services among adolescents.

The gender distribution of the index clients in this study shows 60.6% females and 39.4% males, which is the reverse of the findings in northcentral Nigeria where 60% of the index clients were males and 40% females (13). This may suggest male HIV dominance in the northern parts of Nigeria, which is the reverse in the southern parts. It may also

indicate the health seeking behavior of women in the southwest who may be more proactive and willing to access index testing services. However, the fact that 60.6% of the HIV-positive index clients were females while 58.4% of their partners were males indicates predominantly heterosexual (96.4%) relationship with few homosexual (lesbian) relationships (3.6%).

The distribution of referral methods showed 56% provider referral and 44% client referral. This is similar to the findings from northcentral Nigeria which showed 68.5% provider referral and 31.5% client referral. This underscores the effectiveness of the provider referral method in increasing uptake of index testing services. Also, more than 80% of partners were contacted via index clients by phone. The HIV positivity rate for this study was 20% and all identified HIV positive partners were linked to ART treatment (100% linkage). The linkage rate in our study is better than that reported from a study in Lesotho where 92% of clients were linked to ART (14). In northcentral Nigeria, the linkage rate was also 92% but there was a higher HIV positivity rate of 51%. This may be due to the higher HIV prevalence in northcentral compared to southwest Nigeria.

The only factor associated with HIV positivity rate in the partners was gender, with significantly higher rate in female (28.7%) than male partners (13.8%) ( $p<0.01$ ), but there was no association with age group ( $p>0.05$ ). The HIV positivity rate in partners of male index clients who were all females (26.9%) was also significantly higher than the HIV positivity rate in partners of female index clients who were mostly but not all males (15.5%) ( $p<0.01$ ). This implies that male HIV positive clients are more likely to infect their female partners than female HIV positive clients infect their male partners. This may be due to the differences in the anatomy of the male and female sexual organs, which present higher likelihood of HIV transmission from male to female than vice versa.

## Conclusion:

This study reports 20% HIV positivity yield among partners of clients who accessed HIV index testing services in selected healthcare facilities in Ondo State, southwest Nigeria, with 100% linkage of all identified HIV-positive partners. The positivity rate among the partners of male index clients was significantly higher than the positivity rate among partners of the female index clients. Due to the high positivity yield among partners of the index clients in this study, index testing proved to be a veritable

strategy to increase HIV case detection and linkage to ART. Hence, proper deployment of index testing will be critical to improving ART coverage and achieving epidemiological control.

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

## **Profile of bacterial pathogens contaminating hands of healthcare workers during daily routine care of patients at a tertiary hospital in northern Nigeria**

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**Abstract:**

**Background:** Healthcare associated infections (HAIs) have been recognized as a critical challenge affecting the quality of healthcare services provided. A significant proportion of these infections result from cross-contamination of microorganisms which are often acquired and spread by direct contact with patients or contaminated adjacent environmental surfaces through the hands of healthcare workers (HCWs). The objectives of this study are to profile bacterial pathogens commonly found on the hands of health care workers while routinely attending to patients in the healthcare facility and to determine their antibiotic susceptibility pattern.

**Methodology:** The fingers of the dominant hand of 300 HCWs at the Barau Dikko Teaching Hospital (BDTH), Kaduna, Nigeria, were imprinted on 5% Sheep blood, MacConkey, and Mannitol salt agar plates and incubated at 37°C for 24 hours. Bacteria isolates were identified by Gram staining and conventional biochemical tests. The susceptibility of isolated bacteria to selected antibiotics was determined by the modified Kirby-Bauer disk diffusion method and interpreted using the 2012 guidelines of the Clinical and Laboratory Standards Institute.

**Results:** Bacteria were isolated from the hands of all 300 HCWs, with coagulase negative staphylococci (CONS) being the most frequent (67.0%, 201/300). Other bacteria identified were *Staphylococcus aureus* (23.7%, MRSA of 3%), *Streptococcus pyogenes* (2.7%), and Enterobacteriaceae (6%). The isolates were highly sensitive to ofloxacin 96.7% (290/300), augmentin 87.7% (263/300) and ceftriaxone 87.3% (262/300).

**Conclusion:** This study demonstrates a high rate of contamination of hands of HCWs with potentially pathogenic bacteria, some of which were multidrug resistant. Concerted efforts should be made to implement programs dedicated to improve hand hygiene practices in the tertiary health care facility.

**Keywords:** Hand hygiene, bacterial, pathogen, healthcare workers, healthcare associated infection

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## **Profil d'agents pathogènes bactériens contaminant les mains des travailleurs de la santé lors des soins quotidiens de routine aux patients d'un hôpital tertiaire dans le nord du Nigéria**

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## Abstrait:

**Contexte:** Les infections associées aux soins de santé (IHA) ont été reconnues comme un défi critique affectant la qualité des services de santé fournis. Une proportion importante de ces infections résulte de la contamination croisée de micro-organismes qui sont souvent acquis et propagés par contact direct avec des patients ou des surfaces environnementales adjacentes contaminées par les mains des travailleurs de la santé (TS). Les objectifs de cette étude sont de dresser le profil des agents pathogènes bactériens que l'on trouve couramment dans les mains des travailleurs de la santé tout en s'occupant régulièrement des patients dans l'établissement de santé et de déterminer leur profil de sensibilité aux antibiotiques.

**Méthodologie:** Les doigts de la main dominante de 300 travailleurs de la santé au Barau Dikko Teaching Hospital (BDTH), Kaduna, Nigéria, ont été imprimés sur des plaques de gélose au sang de mouton à 5%, MacConkey et Mannitol et incubés à 37°C pendant 24 heures. Les isolats de bactéries ont été identifiés par coloration de Gram et tests biochimiques conventionnels. La sensibilité des bactéries isolées aux antibiotiques sélectionnés a été déterminée par la méthode de diffusion sur disque modifiée de Kirby-Bauer et interprétée en utilisant les lignes directrices de 2012 du Clinical and Laboratory Standards Institute.

**Résultats:** les bactéries ont été isolées des mains des 300 TS, les staphylocoques à coagulase négative (CONS) étant les plus fréquents (67,0%, 201/300). Les autres bactéries identifiées étaient *Staphylococcus aureus* (23,7%, SARM de 3%), *Streptococcus pyogenes* (2,7%) et Enterobacteriaceae (6%). Les isolats étaient très sensibles à l'ofloxacine 96,7% (290/300), à l'augmentationin 87,7% (263/300) et à la ceftriaxone 87,3% (262/300).

**Conclusion:** Cette étude démontre un taux élevé de contamination des mains des travailleurs de la santé par des bactéries potentiellement pathogènes, dont certaines étaient multirésistantes. Des efforts concertés devraient être faits pour mettre en œuvre des programmes visant à améliorer les pratiques d'hygiène des mains dans les établissements de soins de santé tertiaires.

**Mots-clés:** hygiène des mains, bactérienne, pathogène, personnel de santé, infection associée aux soins de santé

## Introduction:

Healthcare associated infections (HAIs) are a major public health challenge with regards to patients' safety. Approximately 20% of patients on hospital admission contract HAIs in developing countries (1) resulting in prolonged hospital stay, increased healthcare cost, development of antimicrobial resistance, and increased morbidity and mortality (2,3,4). In the United States of America, an estimated 2 million hospitalized patients are affected by HAIs yearly resulting in mortality of 100,000 patients (1).

The major source for transmission of HAIs is the contaminated hands of healthcare workers (HCWs) (5,6). The objectives of this study are to profile the bacterial pathogens contaminating hands of HCWs during routine patient care, and determine their antimicrobial susceptibility pattern.

## Material and methods:

### Study setting

This study was conducted from May to July, 2019, at the Barau Dikko Teaching Hospital

(BDTH), Kaduna, a tertiary healthcare facility and also a large referral institution providing primary and tertiary medical care for residents in the State and environs. The hospital has several units in which member of staff interact and carry out routine daily patients' care. Ethical permission was obtained from the Hospital Research and Ethics committee and informed consent obtained from all study participants.

### Subjects, sampling and bacterial identification

Three hundred healthcare workers were consecutively selected from five major cadres of staff of the hospital (doctors, nurses, laboratory personnel, attendants, and support staff) and medical students, in equal numbers. The five fingertips of the dominant hand of each subject were imprinted on MacConkey, Mannitol salt and Blood agar plates, which were incubated at 37°C for 16-18 hours. Bacteria identification on culture plates was done by Gram staining, conventional biochemicals (catalase, coagulase, triple sugar iron agar, indole, urease citrate, and the Microgen identification kit (Oxoid Ltd, UK). The slide agglutination test with Staphytech (Oxoid Ltd, UK) was used in addition to the con-

ventional biochemical tests for further identification of the staphylococci.

#### Antibiotic susceptibility testing

Antibiotic susceptibility of the isolates was determined by the modified Kirby Bauer disk diffusion method (8) against eight selected antibiotics; ofloxacin 5µg, amoxicillin/clavulanate (augmentin) 30µg, gentamicin 10µg, cefuroxime 30µg, cefotaxime 30µg, ceftriaxone 30µg, erythromycin 15µg, and ampicillin 10µg. Cefoxitin 30µg was used as surrogate for the detection of methicillin resistance in *Staphylococcus aureus*.

A colony of the test organism was picked with sterile wire loop and immersed in peptone water. The turbidity of the suspension was standardized against 0.5 MacFarland standards. The suspension of the organism was streaked with a sterile swab stick on an entire plate of Mueller Hinton agar and the antibiotic disks were placed on the plate using sterile forceps. The susceptibility culture plates were incubated at 37°C aerobically for 16-18 hours while the culture plates for oxacillin resistance testing was incubated for 24 hours. Susceptibility or resistance was determined by measuring the zones of inhibition with a calibrated ruler and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (9).

#### Data analysis

The Statistical Package for the Social Sciences (SPSS) software version 20.0 was used for data analysis.

### Results:

There were 300 study participants with 50 from each of the five cadres of HCWs (doctors, nurses, laboratory personnel, attendants, and support staff mainly administrative officers) and medical students.

#### Bacteria cultured from hands of participants

Bacteria were cultured from the hands of all the study participants (100%). The most common bacterial isolates were CONS 67% (n=201), *Staphylococcus aureus* 23.7% (n= 71), *Escherichia coli* 4.3% (n=13), *Streptococcus pyogenes* 2.7% (n=8), *Klebsiella pneumoniae* 1% (n=3), *Proteus mirabilis* 0.7% (n=2) and *Salmonella typhi* 0.7% (n=2) (Table 1). CONS was most frequently isolated from hands of the support staff with frequency of 20.8% (42/201) and the nurses 19.9% (40/201) while it was least frequently isolated from hands of doctors

13.4% (27/201) and laboratory personnel 10.5% (21/201).

Conversely, *S. aureus* was most frequently isolated from hands of laboratory personnel 33.8% (24/71) and medical doctors 32.4% (23/71), and least frequently isolated from hands of nurses 7% (5/71) and medical students 7% (5/71). The Gram-negative bacteria were most frequently isolated from hands of medical students 40% (8/20) and nurses 25% (5/20) (Table 1).

#### Susceptibility of the bacterial isolates

Most of the bacteria (96.7%) cultured from the hands of the study participants were sensitive to ofloxacin. Sensitivity to other antibiotics were; cefotaxime (76.7%), ceftriaxone (87.3%), augmentin (87.7%), gentamicin (80.3%) and erythromycin (62.3%) (Fig 1). Nine of the 71 (12.7%) *S. aureus* isolates were methicillin resistant.

### Discussion:

The growing awareness of multidrug resistance and the development of 'super bugs' has led to an increasing introspection on the practices in health care facilities which promote the transmission of microorganisms and the role of HCWs in the chain of transmission. Hand hygiene has been identified as a major strategy in reducing the transmission and prevalence of HAIs (10). This study highlights bacterial colonization of the hands of a wide range of HCWs who are constantly caring for patients. These organisms may constitute an infection risk to HCWs and to those seeking care in this healthcare facility.

Our study demonstrates a high rate of hand contamination with CONS and *S. aureus* among the study participants which is consistent with findings of other studies that showed these organisms to be resident and sometimes transient flora (11). Although these organisms have been shown to be non-pathogenic, they have the potential to cause life threatening nosocomial infections in susceptible hosts (10,12).

The least frequently isolated bacteria were *K. pneumoniae*, *P. mirabilis* and *S. typhi* in this study. These Gram-negative bacteria are members of the family Enterobacteriaceae that have the propensity to acquire plasmids which carry genes for drug resistance, and make HAIs caused by these organisms difficult to treat (13,14). They have also been linked with HAIs

Table 1: Frequency distribution of bacterial isolates on the hands of healthcare workers

Organism/ Cadre of HCW	Attendant	Doctor	Laboratorian	Nurse	Others	Student	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
CONS	35 (70)	27 (54)	21 (42)	40 (80)	41 (82)	37 (74)	201 (67)
<i>S. aureus</i>	7 (14)	23 (46)	24 (48)	5 (10)	7 (14)	5 (10)	71 (23.7)
*MRSA	5 (10)	0	2 (4)	2 (4)	0	0	9 (3)
<i>S. pyogenes</i>	5 (10)	0	2 (4)	0	1 (2)	0	8 (2.7)
<i>E. coli</i>	0	0	2 (4)	3 (6)	1 (2)	7 (14)	13 (4.3)
<i>K. pneumoniae</i>	1 (2)	0	0	1 (2)	0	1 (2)	3 (1)
<i>P. mirabilis</i>	1 (2)	0	0	1 (2)	0	0	2 (0.7)
<i>S. typhi</i>	1 (2)	0	1 (2)	0	0	0	2 (0.7)
Total	50 (100)	50 (100)	50 (100)	50 (100)	50 (100)	50 (100)	300 (100)

CONS= Coagulase negative staphylococci; \*MRSA=Methicillin resistant *Staphylococcus aureus* (within *S. aureus* isolates); HCW = healthcare worker

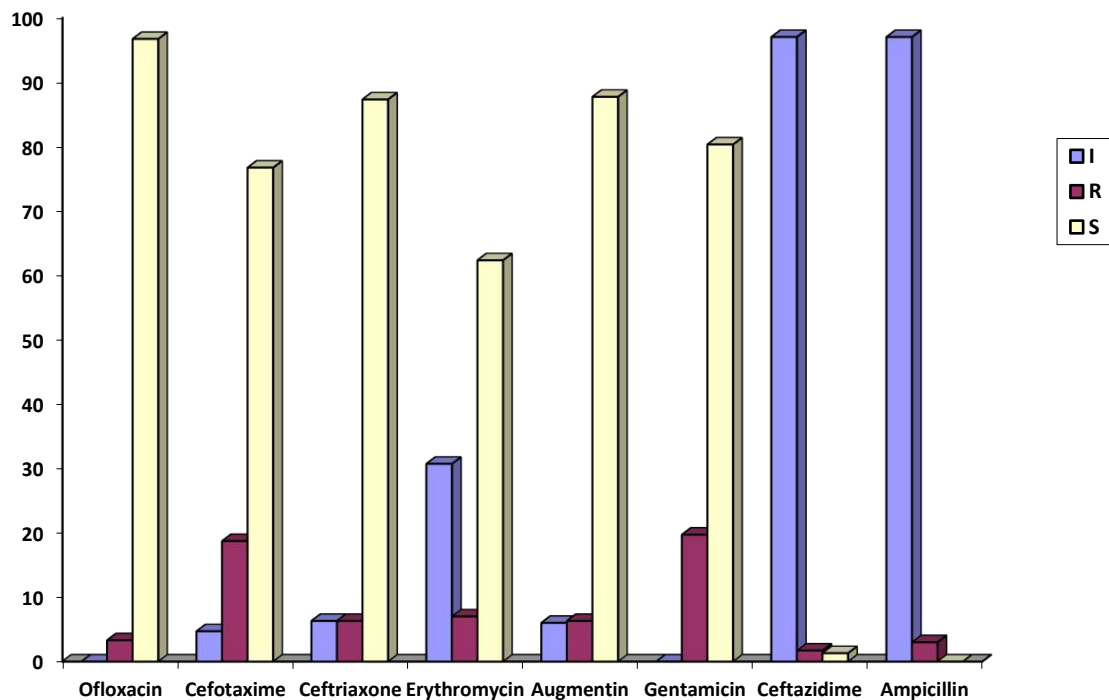


Fig 1: Histogram showing antimicrobial susceptibility pattern of isolated bacteria

following the use of ventilator or urethral catheterization, and after faeco-oral transmission. Sixty-two (87.4%) out of the 71 isolates were methicillin sensitive *S. aureus* (MSSA) while 9

(12.7%) were methicillin resistant *S. aureus* (MRSA). This MRSA rate is lower than the rates reported by Fadeyi et al., (15) and Abdullahi and Iregbu (16).



Some cadres of staff in the study were more frequently colonized with one bacterial than the other for example nurses and support staff were most frequently colonized with CONS while doctors and laboratory personnel were least frequently colonized. Conversely, doctors and laboratory personnel were most frequently colonized with *S. aureus*. Also, medical students and nurses were most frequently colonized with Gram negative bacteria while doctors were least frequently colonized. These findings contrast those of previous studies (17,18).

Poor hand hygiene performance has been documented amongst HCWs in Kano, a city in northwest Nigeria (19). Therefore, our findings may be indicative of low compliance across many health institutions across the country and suggests instituting and implementing policies which govern and guide hand hygiene practices and continuous training across all cadres of HCWs including students. The high frequency of *E. coli* contaminating hands of medical students observed in our study is similar to that of Watutantrige et al., (10). This may be due to poor toilet and hand hygiene practices by the students as postulated by the study.

The high frequency of isolation of *S. aureus* among laboratory personnel is similar to the findings in Guangzhou, China, which showed that medical laboratory staff especially those in the microbiological laboratories had significant exposure to *S. aureus* with increased risk of colonization and infection from contact with patient samples. *S. aureus* contamination of HCWs has previously been linked to the contamination of white coats which act as potential reservoirs of infection and re-infection (20). This may also explain why medical doctors had the highest percentage of carriage of *S. aureus* in this study.

The isolates showed highest sensitivity to ofloxacin, augmentin and ceftriaxone (all bacteria) and least sensitivity to erythromycin (*S. aureus*, CONS), ampicillin and ceftazidime (enteric bacteria). Similar patterns of susceptibility were reported for isolates in the study by Abdullahi and Iregbu (16) with detection of significant resistance of *S. aureus* to penicillin, erythromycin and clindamycin but sensitivity to vancomycin, cefazolin and imipenem.

## Conclusion:

This study demonstrates a high rate of contamination of hands of HCWs by potentially pathogenic microorganisms, some of which are multidrug resistant. Improvement of hand hygiene practices is necessary to minimize the

risk of cross contamination and spread of infectious pathogens in healthcare facilities

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