Nigerian Journal of Clinical & Biomedical Research

Volume 6 Number 1 June 2012 ISSN: 1596-0730

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The Official Journal of
Nigerian Institute of Medical Research

Research for National Health

Nigerian Journal of Clinical & Biomedical Research

The Official Journal of
Nigerian Institute of Medical Research, Yaba Lagos
Email: njcbr@nimr_ng.org **Website:** www.nimr_nig.org

Nigerian Journal of Clinical and Biomedical Research® (ISSN 1596-0730) is apeer reviewed journal that targets scientists , policy and decision makers and the general public .

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Editorial

A Welcome from the New Editor in Chief

Welcome to the first edition of *Nigerian Journal of Clinical and Biomedical Research (NJCBR)* that I have compiled since taking over the reins as the Editor in Chief, on behalf of the new editorial team made of experienced researchers and academicians. This is an exciting time for us working on NJCBR, as over the next few years we will take the opportunity to re-engineer the journal, reorganize the format, content, and access to NJCBR to enable it function in a style that we hope befits modern thinking, research, technology and development.

The content of a journal is the most important aspect of a journal. It is what makes Researchers, scientists and policy makers want to read a journal as well as submit manuscripts. In view of the many journal available today, we want NJCBR to be competitive. We want it be a unique journal that will encourage authors to think outside the box and encourage new ideas. Though we will not be completely changing the format of the journal we will introduce two new sections of Ethics corner, and Research and methods update. While Ethics corner will focus on ethical issues in research, Research methods update will focus on the must know for every scientists especially the upcoming scientists. In this section contributors will be at liberty to format their manuscript to suit their style. Experts in this field will be invited to make contributions, however contribution from uninvited authors will be considered.

As most scientists are reluctant to publish negative research findings, which often contribute to body of knowledge as much as positive findings, we will encourage submission of negative studies, short articles and research letters to help ensure that all data have the chance to be shared with the scientific community.

NJCBR will transition from the current submission style to a more efficient electronic submission system and ultimately the indexing in all major databases.

We hope that these changes and innovation will make NJCBR current and relevant. While these are laudable ambitions it requires dedication and hard work. We know you are key to achieving these laudable objectives. Please help and make recommendation of what you think will assist us to attain the set goals.

I hope you enjoy this edition of NJCBR, and will stay with us all the way. Together we can make the dream come true.

Finally, be assured that every change we will introduce in the near future is with your reading satisfaction and expectations in mind, and with the sole aim of serving you better.

Oliver C. Ezechi
Editor in Chief

Review Article

Is there a place for mandatory HPV vaccination in Nigeria? Public health, human rightsand ethical considerations

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Summary

Some strains of the Human Papilloma Virus are carcinogenic. They are a common sexually transmitted infection and are causally related to cancer of the cervix. Cervical cancer is the commonest genital tract malignancy in Nigeria and is associated with high mortality rates. Vaccination against the HPV protects from the infection and if administered before a young girl becomes sexually active will effectively prevent development of cervical cancer in later years. This paper examines the human rights and ethical implications of a compulsory vaccination programme to adolescents aged about 12 years. A mandatory vaccination programme infringes on the right to self determination/autonomy and liberty of the parents/legal guardian as guaranteed in the Nigerian Constitution. It also appears discriminatory on a gender basis. However the right to life of the adolescent who at 12 has limited autonomy is so fundamental that the Government may override the right of autonomy of the parents to protect her from a direct threat to her life posed by cervical cancer. The ethical principles of beneficence, non maleficence, justice and autonomy (for the child) tend to provide a morally justified basis for a compulsory programme, even though the autonomy of the parents becomes restricted.The final goal of a compulsory vaccination programme – to prevent disease, disability and death is noble and moral both from the deontological and utilitarian perspectives. It is effective but bears human rights and ethical burdens. Distributive justice is better served by a compulsory rather than the alternative voluntary vaccination programme or screening services as the disadvantaged population at a higher risk are reached and the cost: benefit calculations are in favour of a preventive rather than a therapeutic management modality for a full blown malignancy. Nevertheless, the burdens can be lessened by involving the communities and local population from the planning to the implementation stages as well as by fair implementation of the programme.A mandatory HPV vaccination programme for young adolescents greatly restricts parental autonomy and liberty but applying principle-based appeals to moral analysis, such a programme can be acceptable on moral, scientific and public health grounds.

Key words: HPV, cervix, malignancy, mortality, pragmatic

Introduction

The Human Papillomavirus (HPV) commonly infects human beings. Of the 100 strains of the virus known, about thirty are transmitted sexually[1] and the infection is a common sexually transmitted infection world over

About 6.2 million new cases of HPV infection are diagnosed in the United States annually with a prevalent rate of 26.8% among American women[2-4]. Four sero types namely HPV 16, 18, 31 & 33 are known to predispose to invasive cervical carcinoma[5,6]. The American Cancer Society reports 10,520 to 12,000 women in the United States develop cervical cancer yearly out of which 3900 to 4100 will die[1]. The incidence of cervical cancer and associated mortality is therefore low in the United States and this is due to the organized

cervical cancer screening program in place in that country[7,8]. On the contrary, screening services are poorly implemented in most African countries and uptake is abysmal, as a result cervical cancer remains the commonest genital tract cancer in such countries[9].

Oncogenesis is triggered by the incorporation of the genomic materials of the HPV into the immature endocervical cells of the transformation zone in the female[7]. The induced dysplastic changes mature through several changes with eventual transformation into invasive cervical cancer in some fractions of cases[10]. Detection at the pre malignant stages and subsequent management reduce both the incidence and fatality potentials of cervical cancer. This is the basis of cervical screening programmes. Prevention of HPV infection and hence the incorporation of its genomic components into the cervical cells will also provide opportunity for prevention of cervical malignant changes. This is the principle behind vaccination against the HPV.

Health promotion is an utmost goal of public health and emphasizes prevention of disease, disability and premature death[11]. Attempts at realising this goal often times conflict with human rights provisions. Past experiences reveal that occasionally some of the rights of a few may be limited for the good of many, hence some coercive measures including mandatory testing and treatment have been employed[12]. Article 29 of the International Bill of Human Rights also recognizes that certain rights must be restricted to protect the community[13] (UDHR). This paper examines the human rights and ethical issues surrounding a mandatory HPV vaccination in Nigeria

The Nigerian situation: Nigeria, the West African country in sub Saharan Africa with a population of over 140 million people[14] is easily the most populous nation of blacks in the World. The population is young with females comprising almost half of the population. This implies that more than 50 million females are at risk of developing cervical cancer in their life time in Nigeria. The Nigerian Health system is poorly organized with inequitable distribution of health care facilities and personnel in favour of the urban centres to the detriment of the rural settlements which houses more than half of the population[15]. With sub optimal budgetary allocation to health less than 5% of the National GDP[15], and access to medical care primarily dependent on user-fee purchasing capability in a dominantly poor population, formal healthcare is not accessible to majority of the population. The high patronage of alternative unorthodox/traditional medical practitioners coupled with prevalent superstitious beliefs and mythical interpretation of medical pathologies complicate the matter.

Nigerian women are heavily burdened with gynaecological diseases including genital tract cancers. Documentations from different centres in the country indicate that cervical carcinoma is easily the commonest gynaecological cancer among Nigerian women who mainly present in the later stages of the disease with consequent high mortality rates[9,16]. Screening for cervical cancer using the Papanicolaou cytological examination of exfoliative endocervical cells is opportunistic, erratic and ineffective in the country. As a result, this preventable malignancy remains common. Late presentations mean that effective surgical procedures are impossible and where radiotherapeutic management techniques are limited and largely inaccessible, the attendant high case fatality rate becomes inevitable.

The introduction of the HPV vaccine which can prevent infections with the carcinogenic strains of the virus and its administration to females at risk would be a logical measure to effectively prevent cervical cancer and the consequent high death rate among Nigerian women. This will go a long way in reducing the burden of disease in women[17]. To be efficient this vaccination has to be delivered before the commencement of sexual activities which can predispose to infection with HPV. The age at sexual debut among girls in Nigeria has been reported to be about 15 years[18]. Proponents of the HPV vaccination programme advocate the administration of the vaccine to girls at about the age of 12 years. Making this laudable programme mandatory however raises human rights and ethical problems.

Human rights consideration: The fourth chapter of the Nigerian constitution[19] guarantees protection of fundamental human rights to her citizens. Essential to the debate on mandatory HPV vaccination to female adolescents about the age of twelve are the rights to personal liberty and autonomy, right to freedom from discrimination and the right to life.

Right to personal liberty: A mandatory vaccination programme implies that neither the assent of the adolescent nor the consent of her parents/legal guardian is to be sought before the vaccine is administered to her. This definitely is a check on their rights to liberty and autonomy as guaranteed in the constitution. Autonomy and right to personal liberty mean that an individual reserves the right to take decisions affecting her in all issues including right to receive or reject any form of healthcare, preventive or therapeutic. It

could therefore be argued on this basis therefore that mandatory vaccination is illegal and not to be justified irrespective of the intended outcome.

Right to freedom from discrimination: The HPV is a sexually transmitted highly infectious diseaseof humans. It therefore results from sexual exchanges between males and females and the female acquires the infection from her male consort. A male vaccinated against the virus would therefore be protected against the infection and would not be able to transmit it to the female who will consequently be protected from genital tract cancers. The carcinogenic forms of the HPV are known to cause cancers of the cervix, vagina and vulva in the female as well as cancer of the penis in the males[20]. A mandatory HPV vaccine for the females only, then appears discriminatory and an infringement on the females' right of freedom against discrimination and would therefore not be justified.

Ethical considerations: All the ethical principles of autonomy, beneficence, non maleficence and justice[21] are worth consideration in a mandatory HPV vaccination programme.

Autonomy: The principle of autonomy demands that each individual be treated as an end in herself, never to be used as a means[22]. It recognizes a competent individual's right to self determination[23]. An individual reserves the absolute right to determine what happens to her or not including vaccinations. For the minor, this autonomy resides with the parent or legal guardian. Mandatory vaccination is therefore an affront on autonomy as it negates whatever the protestations of the parents and the voluntariness that should follow informed consent[24].Moreover, genital cancer affects an individual and does no direct harm to a third party, in such a case; an obligatory vaccination programme may be deemed unacceptably paternalistic[24]. The argument that such vaccination programme may provide requisite herd immunity[25] against the HPV may be interpreted as using the individual as a means which will not be acceptable. On the other hand, one may argue that the adolescent's right to obtain preventive measure far outweighs the respect for parent's autonomy because the danger to health involves the life of the adolescent directly[26].

Beneficence: The ultimate aim of a mandatory HPV vaccination programme is to prevent the development of cervical cancer in females, prevent death and promote health. This is a noble objective and would therefore justify the programme on a beneficence scale – preventing harm and doing good. It is further argues that the ethical principles of beneficence and non maleficence and the desire to prevent harm are weighty enough to override the principles of autonomy and liberty[26]. The Philosopher John Stuart Mills on liberty noted that 'the only purpose for which power can be rightfully exercised over any member of a civilised community against his will is to prevent harm"[27].

Non maleficence: There has been no scientific evidence that a HPV vaccination brings about any physical harm to the individual though the long term effect is not apparent at the present[8,28,29]. One may take the argument further that a psychological harm may be inflicted on the parent/legal guardian whose authority, liberty and choice is undermined and this could impair citizen's trust in public health care system[26]. One needs to balance these arguments to arrive at a consensus and one may also view opposition to the benefits of vaccination as maleficence[26].

Justice: Cervical cancer can be prevented given an effective screening programme as obtainable in the developed countries. The screening programmes are simple and affordable with less economic implication for the society. HPV vaccines at present remain costly but evaluated against the financial cost and psychological burden of a full blown cancer would be considered more economically viable with a favourable cost: benefit ratio and therefore just – distributive justice.

Arriving at a conclusion – a pragmatic approach: The public health goal of HPV vaccination, preventing incidences and related deaths from malignancy of the genital tract is laudable. Viewed deontologically, this motive is noble and morally justified and the consequent prevention of disease, disability and death for the majority will also justify it in the utilitarian context. The contest therefore is not on the nobility and morality of the vaccination but on its mode of implementation: making it mandatory for adolescents (with limited autonomy) about the age of 12 years probably not sexually active yet. Opponents mainly parents/legal guardians argue that compulsory vaccination erodes their autonomy, liberty and freedom to choose what they deem best for their daughters. Proponents however point to historical successes achieved through mandatory vaccinations like in the cases of Poliomyelitis (Polio), measles and small pox[26]. In 1905 a Supreme Court ruling in *Jacobson versus Massachusetts* declared a compulsory vaccination programme against small pox legal[26]. However opponents are quick to point out the dissimilarities between Polio and the HPV. Though

both are contagious infectious diseases, Polio is transmitted faeco – orally while HPV is contracted sexually between persons[26], they posited that a mandatory HPV programme might undermine sexual morality among adolescents and compromise family values which emphasize abstinence and abhor premarital sexual exchanges[26]. Such parents would prefer a voluntary programme. A further argument is that the HPV vaccination may confer a false sense of security of being protected against all forms of sexually transmitted infections[30-33]. One needs to evaluate the programme pragmatically considering the following:

The final goal: The ultimate objective of a mandatory vaccination against HPV is to reduce morbidity and mortality from cervical cancer; this is immutable and morally acceptable. From the public health angle if this goal is achieved for the majority, even when it involves infringing on some individuals' rights, it will be a morally permissible action for the benefit of the community (communitarianism). An opposing view would be that an efficient cervical screening programme would appear cheaper and still achieve the same end of reducing disability and death from cervical cancer. At risk females being urged through education to uptake the services freely and voluntarily with informed consent. This may appear plausible since cervical cancer bears no direct harm to a third party. In Nigeria the reality remains that cervical screening services are not organized, are not widely available and uptake very minimal, consequently, the incidence of the disease and deaths arising therefrom have remained overwhelming. A compulsory vaccination programme may be more realistic considering the successes recorded with prior vaccination programmes against Smallpox, Polio and Measles Tetanus.

Effectiveness of HPV Vaccination: Scientific concerns have been raised on the long term effect and efficacy of the HPV vaccine[8,28]. To the best of my knowledge, the vaccination has not been evaluated in any scientific literature in Nigeria. However, literature from the United States considers the vaccine to be very effective[8,28], given in a three-dose series over six months is said to be very valuable in protecting against infections by the four carcinogenic strains of the HPV in females who have not been previously exposed to HPV[34]. On the contrary, opponents argue that screening for cervical malignancy has reduced mortality by 80% and that a mandatory vaccination can only but add a little benefit[8]. Putting all together, one believes that even a marginal benefit would translate to some lives saved and this margin can be gained through compulsory vaccination made universally available in Nigeria where deaths from the disease remain evidently and unacceptably high.

Known or potential burdens: For all intents and purposes, a compulsory vaccination programme bears human rights and ethical burdens. These mainly revolve around autonomy/personal liberty, discrimination and justice.

A compulsory programme no doubt restricts the autonomy and personal liberties of parents who might prefer otherwise for their daughters in a voluntary programme. Such parents would suggest abstinence education and practices to preclude infection with HIV and maintenance of family values of sexual morality. It must however be pointed out that a compulsory programme does not deprive the parents of the opportunity or rights to inculcate their own values to their off springs[26]. Here, the burdens of disease morbidity and mortality from cervical cancer for both individuals and the community appear to overshadow the benefits accruable from upholding parental rights and authority[26]. Furthermore, adolescents are a vulnerable group and reserve the right to be protected against vaccine-preventable illnesses and the society has the legitimate duty to safeguard their wellbeing which might be compromised by the choices of their parents/guardians[24]. The society is ought to take special measures to protect those with limited autonomy[11].

The right not to be discriminated against is one also thought to be compromised by a mandatory HPV vaccination. This programme targets only females whereas HPV infection affects both females and males alike and can be transmitted in both directions. Moreover HPV infection is also linked to penile cancer in the males[20]. Females may feel discriminated against and possibly stigmatized by this practice. The males on the other hand may complain about being left out of this preventive effort. However, it must be argued that incidence of cancer of the penis is so minimal compared with cervical cancer which poses real threat to women's health and survival. The causal relationship between sex and HPV infection is one of the pivotal arguments against a compulsory programme. It is yet to be ascertained if such opponents would stand against mandatory vaccination schemes against Polio, Measles and the like highly non sexual contagious diseases. Limiting opportunity to vaccination because of age, social views about sexual behaviour and mode of transmission can be considered discriminatory[26] and not acceptable.

Considerations of causes of death should never be the determining factor for access to health care regardless of whether human behaviour can be implicated[26].

Finally is the issue of justice. Rawl's principle of Justice considers it injustice to provide health care to one group and withholding same from another for reasons of age, race, gender, socio economic standing, religion or other factors[35], it is therefore just to provide universal HPV vaccination against the exigent ideals of sexual activity among the youth[26]. Cervical cancer predominantly affects the poor in Nigeria and compulsory vaccination with cost borne by the Nigerian Government would be the most effective means of protecting the poor and disadvantaged from this scourge[36]. A voluntary vaccination programme will probably preserve the disparity between advantaged and disadvantaged populations and the groups at the greatest risk of HPV infection and cervical cancer. Social justice would be served by a mandatory vaccination programme providing the greatest utility for the people who are at the greatest risk of the disease[26]. However, the cost-benefit implication must be analysed to situate it properly in the Public Health context.

Minimizing the ethical burdens/alternative approaches: The question arises, how possible the human rights and ethical burdens can be minimized. The importance of community involvement from the planning stages through the implementation phases cannot be overemphasized. Such community participation puts the community in good stead to appreciate the importance of the programme and be more amenable to it as their views would be taken into consideration and their fears allayed. This will greatly lessen the human rights and ethical burdens of a mandatory vaccination programme. Meanwhile, it would be easy to assume that alternative approaches of a voluntary vaccination programme and/or cervical cancer screening services can minimize the human rights and ethical burdens implicit on a compulsory vaccination programme by their voluntary nature thereby re instituting the autonomy and respect for persons for the parents/legal guardians who may not want to give in to mandatory vaccination of their daughters. A further concern would be the effectiveness of these voluntary programmes. Compulsory vaccination confers immunity and consequent protection universally among the population whereas voluntary programme may not provide enough herd immunity in a community. One may however posit that with adequate public health education and mobilization, uptake of voluntary vaccination and/or cancer screening services may be optimized and maximized to ensure a near universal protection. This should be the challenge for public health professionals.

Fair implementation of the programme: A fair programme implementation will ensure distributive justice in relation to the burdens and risks of the programme in Nigeria. For this programme to be morally justifiable it must be fairly implemented to bring about adequate protection against cervical cancer across all regions and population groups in Nigeria far above the harms of erosion of parental autonomy and liberty.

Balancing the risks and benefits of the programme fairly: Compulsory HPV vaccination bears the human rights and ethical burdens of relegating informed consent, restricting autonomy and liberty and connoting discrimination against the female population. HPV is highly infectious, causes cervical cancer among other genital tract cancers and is associated with high mortality rates resulting in an unbearable disease, disability and death burdens for the Nigerian woman and society[20]. HPV vaccination prevents all these. The ethical principles of beneficence, non maleficence, autonomy (for the vulnerable adolescent) and justice implicit in the mandatory vaccination programme are strong enough to justify it[26].

Conclusion

Historical evidence seems to justify mandatory vaccination programmes as seen with the successful eradication of smallpox and effective control of some very contagious infections like Polio and Measles. Malignant gynaecological diseases place huge burdens on women's health in the developing countries including Nigeria where screening programmes have been largely ineffective. Such malignancies have been conclusively aetiologically linked to HPV infections[6,20]. Vaccinating young girls about the age of 12 years before they get sexually active against the HPV would reduce the incidence of cervical cancer and the associated mortality. A compulsory vaccination programme as against voluntary programme infringes on the human right exercises of parents/guardians in terms of autonomy and liberty and may also appear as discriminatory against the females. However if fairly implemented and balanced against the fundamental human right of the child to life and the ethical principles of beneficence, non maleficence and distributive justice, it may seem justified on moral, scientific and public health basis – though this can be contested. Involvement of the communities from the planning to the

implementation aspects of the programme will go a long way in reducing the attendant human rights and ethical burdens inherent in a compulsory vaccination programme.

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Review Article

Reducing HIV transmission through health promotion in Nigeria

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Summary

Human Immune Virus (HIV) is a health condition that continues to pose a threat to human existence. The major transmission route includes sexual transmission, mother to child transmission (MTCT), sharing of HIV contaminated sharp objects and blood transfusion. This may be fuelled by various reasons such as lack of awareness; HIV associated risky behavior, lack of health promotion and poor leadership. Nigeria has proffered strategies to reduce the burden of HIV in the country. However there is still the need for the right policies to be put in place to tackle the HIV crisis. In an effort to control the emergence of new cases as well as manage the existing infected population, health promotion strategies were introduced in Nigeria. Some of these strategies include HIV counseling and testing, proving antiretroviral therapy, PMTCT, increasing awareness. Means of implementing the strategies that could effectively reduce HIV transmission in Nigeria were suggested.

Keywords: HIV Transmission, Health Promotion

Introduction

Health promotion is defined as a comprehensive process of enabling people increase control over the determinants of health. It involves the social, economic and physical environment[1].The resultant effect is an improvement in health and the reduction of the burden of Human Immuno-deficiency Virus (HIV) and other sexually transmitted disease in general. HIV is a disease condition that poses a threat to human existence[2], unfortunately practices that promote the infection still abound in Nigeria. This is because both individuals and the government are lacking in their role to subdue the spread of the virus.

Nigeria lacks the political leadership required to deal with issues arising from the HIV epidemic because the leaders are not committed to improving the health care system and tackling HIV. The Nigerian Government has embarked on strategies aimed at reducing the transmission of the virus.[3] However, for an effective implementation of these strategies, there is a need to address the issue of poor leadership and mismanagement of funds provided by the government and donor partners for the treatment of HIV.

This article will give an overview of the HIV pandemic in Nigeria and will examine the health promotion strategies that have been put in place in Nigeria to address transmission of the virus. Lastly, the article will recommend methods that can be incorporated into improving quality of care for HIV patients and reducing HIV transmission in Nigeria.

Overview of HIVinfection in Nigeria

HIV was first diagnosed in Lagos, Nigeria in 1986[4], since then the HIV prevalence rate has increased from 1.8% in 1991 through 4.5% in 1996, 3.8% in 2001, 5.0% in 2003 and 4.4% in 2005 to 4.6% in

2008[5,6]. However, the prevalence rate seems to have stabilized with a prevalence rate of 3.6% in 20097. Nigeria has a population of about 127 million people and approximately 6.1 million people are living with HIV in the country, making Nigeria the country with the third highest number of infections in the world and second in Africa[8,9]. The human toll of HIV has been experienced by families, communities and the nation at large[10]. In addition, it has also become the major cause of illness and death among young and middle aged adults, depriving households and society of essential human resource[10]. Various means of HIV transmission range from unsafe and unprotected sexual intercourse, infected mother to child transmission, transfusion of infected blood, the sharing of HIV contaminated needles and sharp objects. However HIV is spread primarily through heterosexual sex in Nigeria[11].

Transmission of HIV in Nigeria Sexual transmission: Heterosexual transmission accounts for over 80 percent of HIV infections in Nigeria[12]. Little information has been provided with regards to homosexuality as this is not openly practiced in Nigeria due to societal and religious factors, as well as illegality of homosexuality by the laws of Nigeria[9]. Nigeria's HIV/AIDS epidemic is perceived as a generalized epidemic, but there are concentrated epidemics among high-risk groups such as men who have sex with men and female sex workers[13]. According to the recent HIV/STI (sexually transmitted infection) Integrated Biological and Behavioural Surveillance Survey (IBBSS), high risk groups are estimated to contribute to 23% of new infections[14]. Multiple sexual partners and low condom use are factors that also contribute to the epidemic in Nigeria[9]. In a study carried out bySamuels and Blake[15] it was observed that women aged 15-49 had fewer sexual partners in their lifetime (1.6) than men (4.3), but of those with multiple partners, only 23% of women and 33% of men use condoms. While most religion play a big impact in promoting abstinence, practices like polygamy, condemning of condom use and the limitation of information and education to women, will only propagate the transmission of HIV[16,17].

Government should always put religion into consideration, especially where women may be limited by their religion to healthcare and information, hence there should be special centres established for HIV education, treatment and care which should be provided by only female health workers to avoid the disobedience of such religious laws. People should be educated about the existence of the virus and its mode of transmission to re-orientate their individual and cultural perception. Schools and local governments can incorporate sex education programs to inform adolescents and individuals about the modes of HIV transmission, this will also reduce risk factors such as unprotected sex and abortion[18].

Mother-to-child transmission: Studies have shown that women are particularly affected by HIV. Of the 3.6million people living with HIV/AIDS in Nigeria, 1.9 million which is about 57% are women[19]. Each year around 57,000 babies are born with HIV because of a lack of awareness of their mothers HIV status and limited access to health care services[20]. There is also the issue of pregnant women detecting their HIV status late. This can be prevented if there are more antenatal clinics spread all over the country which will easily be accessible by these women. It is estimated that 220,000 children are living with HIV in Nigeria, most of who became infected from their mothers at birth or through breastfeeding[21].

Blood transfusion: HIV transmission through unsafe blood accounts for the second largest source of HIV infection in Nigeria which may be as a result of an increasing demand of blood for emergency cases, some illegal blood banks. Most hospitals in Nigeria are unable to meet the demand for blood, therefore there is a risk of using contaminated blood when purchased from commercial blood banks[23].

Sharp Objects: Intravenous drug users who share needles are also involved in the transmission of HIV. Sharing of unsterilized sharp objects such as blades and needles with infected persons at salons during manicure, pedicure and fixing hair extensions are other notable ways of HIV transmission[24].

Leadership and health promotion in Nigeria:
Nigeria has continued in her attempt to reduce the burden of HIV in the country but lacks the right leadership to tackle the HIV crisis. This is because there is a high level of corruption and the leaders are not committed to improving the standards of living of the citizens[25]. The lack of functional health care facilities have provided the citizens with no option but to access HIV treatment and care from private hospitals who have capitalized on this opportunity by becoming profit making ventures[26]. However, to effectively curtail the virus and the increase in transmission, the leaders

have to show a level of interest and commitment by providing funds to implement various projects and appointing professionals who are passionate in effecting changes in the health sector.

In an effort to reduce the impact of the pandemic, Nigeria has implemented several control and intervention strategies to manage those that are already infected with the HIV virus and prevent the emergence of new cases[27]. Adopting health promotion in Nigeria was to improve health care practice within the Nigerian Health System over the years[28]. In addressing the weakness of the health system, Nigeria embarked on a health system reform process with thrusts on the following strategic areas:

1. Improving the stewardship role of Governance
2. Strengthening the National Health System and its management
3. Improving availability of Health Resources and their Management
4. Reducing Disease Burden
5. Improving access to Quality Health Service
6. Promoting effective Partnership, Collaboration.

According to FMOH report[29] the National health promotion policydraws significantly from the National health policy which identified ten actions by individuals, families, communities and governments as being essential for the promotion of health. One of the actions that is directly related to this essay is the "Adoption of Measures to prevent the spread of HIV and promote reproductive health[29]".Though the measures prior to the Health Promotion policy have been adopted to alleviate the impact of the HIV epidemic, provisions in the policy will further improve these strategies.

Interventions to reduce HIV transmission

There are various strategies that are can be implemented to reduce HIV transmission in Nigeria. Presently, some of these strategies are being implemented while some others such as palliative care and home based care are still in need of improvement. Some strategies to reduce HIV transmission include:

1. HIV Counselling and Testing (HCT)
2. Providing Antiretroviral therapy
3. Ensuring adherence to Highly Active Antiretroviral drugs (HAART)
4. Prevention of Mother to Child Transmission (PMTCT)
5. Improved blood Safety
6. Palliative Care, Home Based Care, Support for Orphans and Vulnerable Children (OVC) as well as adequate treatment of STIs.
7. Increasing Awareness
8. Increasing the use of Condoms

HIV counselling and testing should be made accessible to all and HCT should consist of pre test counseling where health care professionals will provide people with information about HIV, its transmission and how it can be prevented. After the HIV test, they should also be engaged in post test counseling. Persons that test negative to HIV should be counseled on positive living and told to repeat the test in 6 months, while those whose results are positive should be counseled about accepting their new status and living positively with the virus[30]. Also, they should be referred to a HIV clinic or hospital for treatment.

This will create an awareness of the existence of the virus and will enlighten individuals about staying protected and reducing transmission. However, in Nigeria there is a distinct lack of HCT centers. In 2007, only 3 % of health facilities had HIV testing and counselling services, 11.7% of women and men had undergone HIV testing and found out the results. In 2008 there was only one HIV testing and counselling facility for every 80,000 Nigerian adults, which shows the need for the government to provide more HCT centers[31].

Furthermore, the lack of HCT centres is also complicated by the standard offered at the few available ones. Iwuagwe and Durojaye[32] are of the opinion that health care facilities offering HIV testing in Nigeria do not follow international standards with regards to confidentiality and ethics.In a study, over half of the people living with HIV reported that they did not know they were being tested for the virus and around one in seven health care professionals admitted that the clients did not give their informed consent. Health care providers should ensure that clients give their informed consent before conducting HIV screening, with confidentiality and anonymity always respected[33].Therefore, they should be trained on this and health care professionals who breach the code of conduct should be sanctioned.

Prevention of mother-to-child transmission of HIV(PMTCT)

In the quest to reduce HIV transmission from a positive mother to her unborn child the Nigerian government has initiated programmes to treat and manage HIV infected women[19].

Access to antenatal clinics should be made available to pregnant women all over the country; pregnant women who register for antenatal clinics

should be educated about HIV and should be screened with their consent. Also positive pregnant women should have access to Highly Active Antiretroviral drugs (HAART) while HIV positive women who are not yet eligible to start antiretroviral drugs should be placed on prophylaxis to reduce the risk of transmission to the baby. Equally positive mothers should be educated about the risk of breastfeeding and information about breast milk substitutes or exclusive breast feeding while on HAART should be provided. The government should subsidize the price of breast milk substitute so that everybody can afford it. Very importantly, health care workers and mid wives should be trained on safe delivery practices[30].

Highly active antiretroviral therapy

These include the use of HAART of various regimen types to increase the patients CD4 count and reduce the viral load[34, 35]. This helps in HIV positive persons to improve quality of life and reduces infectivity. Also, in a sero discordant couple, a positive partner who has been adhering to ARV will have a high CD4 count and a low viral load therefore reducing the risk of infecting the negative spouse.

Despite the importance of HAART, a lot of individuals are scared of the side effects of the drugs such as lipodystrophy, neuropsychiatric disorders and do not adhere to the drugs[36].Another reason for defaulting in their drug regimen includes fear of stigma and being rejected by their spouse, family, friends or colleagues who might see them taking the drug. As such, great adherence counseling is involved in HAART use; therefore the Government and every HIV clinic should take this very importantly.

Condoms

Proper use of condoms helps to reduce the rate of HIV transmission.[37] Condoms can be expensive to afford by many Nigerians therefore international organizations have intervened by providing condoms to health care centers which in turn distribute to patients free of charge. However, the total number of condoms provided by international donors has been relatively low. The average number of condoms distributed in Nigeria by donors was 5.9 per man; per year. 75 percent of health service facilities visited in a survey did not have condoms or contraceptive supplies[38]. Restrictions on promoting the use of condoms have hampered HIV prevention efforts. For example, a radio advertisement was suspended by the Advertising Practitioners Council of Nigeria (APCON) for promoting messages that suggests that it is acceptable to engage in premarital sex as long as a condom is used.The number of female condomssold in Nigeria has significantly increased from 25,000 in 2003 to 375,000 in 2006[39]. Individual perception and beliefs about the use of condoms should be broadened through education and enlightenment to reduce unwanted pregnancies, HIV and STIs in general. Condoms should be made accessible to individuals who are of age to purchase it. Also female condoms should be provided at a subsidized rate and people should be taught how to use both the male and female condoms through regular condom demonstration by the health workers in health care centers.

Education

Positive living entails practicing safe sex, healthy diet, regular exercise, adhering to antiretroviral drugs and keeping to the doctors and counselor appointment. Culturally, the issue of sex is a very private subject in Nigeria and the discussion of sex with adolescents is often seen as inappropriate. Cultural and religious beliefs have hindered attempts to create awareness by providing sex education for young people[40].In 2009 only 23 percent of schools were providing HIV education to students, and 25 percent of men and women between the ages of 15 and 24 could identify ways of preventing sexual transmission of HIV[20]. In some regions of Nigeria girls marry relatively young, often to older men who already have wives. Studies have shown that those who are married at a younger age have less knowledge about HIV than unmarried women, and are more likely to believe they are low-risk for becoming infected with HIV. Therefore, HIV education initiatives need to focus on young married women, especially as these women are less likely to have access to health information Clients should also be educated about positive living to enhance their CD4 counts and reducing their viral load.

Media campaigns

Nigeria is a large and diverse country, therefore educating and raising awareness about HIV through media campaigns are a practical way of reaching people in different states. Radio campaign like the one initiated by the Society for Family Health in Nigeria have been successful in imparting knowledge and changing behaviour. "Future Dreams", was broadcasted in nine languages on 42 radio stations. The program focused on encouraging consistent condom use, increasing knowledge and increasing skills for condom negotiation in single men and women between the ages of 18 and 34. In 2005, a campaign was launched to increase public awareness of HIV. This campaign capitalized on

the increase of mobile phones owners and sent text messages with information about HIV/AIDS to 9 million people. Another high profile media campaign is fronted by Femi Kuti, the son of Fela Kuti, the famous Nigerian musician who died of HIV/AIDS in 1997. He appears on billboards throughout Nigeria with the slogan 'HIV/AIDS no dey show for face', which means you cannot tell someone has HIV/AIDS by looking at them. However, there is a need to create more awareness in the remote areas of the country. Also, advertisements and media campaigns should be done in the local dialects rather than English to ensure that everyone understands the information being passed.

Government should ensure sterilizing kits as safety requirement at salons, tattoo shops and dental clinics[41]. Individuals should also be encouraged to buy their own clippers, manicure and pedicure sets which they can take along when going to the salon and if they cannot afford it, they should insist that the barber or dentist sterilizes the equipment in their presence[42]. The Nigerian Federal Ministry of Health have responded by backing legislation that requires hospitals to only use blood from the National Blood Transfusion Service, which has improved the safe blood-transfusion in Nigeria.

Recommendation

Health promotion campaigns should focus on health care workers, traditional healers, traditional birth attendants, religious leaders, families and people living with HIV. By providing a detailed explanation of the virus, its transmission routes, treatment and management in order to improve control effectively[50].Introducing comprehensive health promotion campaigns through the mass media and providing community-based programs to reach people at the grassroots to control the HIV epidemic will also go a long way. Health care providers should work in line with their ethical principles or standard operating procedures where information will be handled privately and utmost client confidentiality must be kept[51].Policies should be put in place restricting people such as healthcare providers, employers, relatives from breaching confidentiality rules.

Government should also introduce voluntary counseling and testing centers in all the local governments in Nigeria to encourage people to go for regular HIV screening and know their status[52].Also incorporating antenatal care departments in hospitals and HIV centers will provide easy accessibility to this care. These centers should have subsidized rates to meet everybody's needs. Home base care programmes can also be implemented to meet the needs of patients who are financially incapable of going to the hospital regularly to pick up their drugs. HIV Support groups should be initiated where PLWHA can share their problems and ideas with others. Anti-AIDS projects should be initiated to ensure safer behavior and practices in ways that are culturally acceptable by the people[53].

Conclusion

In Nigeria, HIV is transmitted through all the methods that have been highlighted in this article, with unprotected sex being the most common form of transmission. Hence the Nigerian government introduction of health promotion policy with strategies to reduce transmission among infected individuals and prevent new infections is very laudable. However, an effective implementation of these strategies can be limited as a result of ineffective leadership, mismanagement of funds, financial constraints, limited access to health care facilities a lack of HIV awareness and education. It is therefore important to be culturally and religiously aware when implementing these strategies by tailoring them to suit the Nigerian society.

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Nigerian Journal of Clinical & Biomedical Research (Jun. 2012) Vol. 6 No.1 19 - 27

Review Article

The Role of Molecular Biology in the Diagnosis and Treatment of Infectious Diseases

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Summary

The burden of Infectious diseases is a global phenomenon. Diagnosis and risk management of infectious diseases depends on accurate diagnosis using variety of methods and this leads to the reduction in mortality and morbidity rates of preventable infections. In modern medicine, molecular biology has moved diagnosis to faster level for rapid detection of infectious diseases using specialized techniques and this is gradually replacing the time consuming less specific methodologies. These techniques are principally used in analyzing molecular basis of biological activities of life. Variety of molecular biology–based tests are able to detect single pathogens, multiple syndrome related pathogens, associations between infectious and non infectious diseases and drug resistance. This review provides information on the understanding and uses of molecular biology in diagnosis and treatment of infectious diseases, particularly those that cause global health problems. It gives an insight into the application of molecular biology to studying all aspects of infectious agents, diagnosis, monitoring, epidemiology and treatment.

Keyword: molecular biology, diagnosis, treatment, infectious diseases.

Introduction

Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi and can be spread, directly or indirectly, from one person to another[1]. They accounted for the death of more than 8.7 million people worldwide, with 63% of all childhood deaths and 48% of premature deaths[2,3]. Most deaths are caused by epidemic infectious diseases such as cholera, meningococcal disease, and measles[4]. Others includes HIV/AIDs, Tuberculosis, Typhoid fever, Staph infections, malaria, *Helicobacter pylori*. Poliomylitis, leishmaniasis and sexually transmitted diseases and respiratory infections[4,5] .

Effective diagnosis and treatment of infectious diseases relies on accurate identification of the implicated pathogenic species using appropriate diagnostic tools. Traditional diagnostic methods are primarily phenotypic methods that include microscopy, staining properties, growth on different laboratory conditions/media, and analyses mainly the characteristic biological proteins. Molecular biology looks at the biochemistry, genetic and protein properties and detects variable morphology of individual cells at the level of nucleic acid[6]. The name molecular biology was coined by Warren Weaver of the Rockefeller Foundation in 1938 and since then it has been an innovative tool in the diagnosis of infectious diseases[7]. In clinical medicine, analyzing molecular basis of biological activity in disease and health conditions plays significant role in the diagnosis and treatment of human infections. Recently, these techniques have assisted pathologist in distinguishing between different infections that have similar symptoms but may require administration of different treatments[8].

Molecular biology methods are more specific, fast, reliable, easily quantified and standardized than phenotypic methods[6,8,9]. These techniques are applied globally in the diagnosis of bacterial, fungal, viral, parasitic infections, drug discovery as well as cancer gene therapy especially through the area of biotechnology[8,10]. Analyzing biological properties at nucleic acid level involves isolation, characterization, sequencing of deoxyribonucleic acid (DNA) ribonucleic acid (RNA) and proteins[10-

[12]. In order to achieve this, several techniques are available. Method earlier described were Polymerase chain reaction (PCR) and PCR- based techniques, others includes detection of protein through Immuno histochemistry, Immunoblotting, Antigen-capture enzyme-linked immunosorbent assay (ELISA) Proteomics and Bioinformatics networking[10-13]. With improved technology, these techniques have moved to higher sensitive, specific, cost effective nanotechnology particularly isothermal nucleic acid amplification method, loop-mediated isothermal amplification (LAMP) and Microfluidics-based diagnostic techniques [10-13].

Molecular biology brought remarkable changes in diagnosis by facilitating rapid, sensitive microbial surveillance and differential diagnosis of infectious diseases. With these methods, unidentified, misdiagnosed or apparently neglected infectious conditions are now recognized thereby reducing rates of transmission, morbidity and mortality[14]. It has also revealed links between infection and chronic diseases, as well as defining human microbiome and its association with disease and health conditions[15-17]. Molecular biology has great prospect in point of care diagnosis, detection of infectious diseases, monitoring and management.

Molecular biology techniques applied in clinical and laboratory medicine: iagnoses of infectious diseases in the field of molecular biology require nucleic acid-based analysis using standard methods for isolating nucleic acids from organisms and clinical material and application of restriction endonulease enzymes, gel electrophoresis, nucleic acid hybridization techniques and DNA microarray technology to analyze DNA or RNA[8,18]. Available literatures have shown that Polymerase chain reaction (PCR) is a central technique in molecular biology. This process was first described by KjellKleppe[19] and Gobind Khorana[20] but was fully developed by Mullis in 1985[21]. Since invention, it has led the way into new era by allowing rapid detection of microorganisms that were previously difficult or impossible to detect by traditional microbiological methods.

Improvement in PCR based technology resulted into the advent of various other methods such as multiplex PCR, real-time PCR and PCR based automations[6,8,22]. Presently clinical applications of innovative technologies such as real-time quantitative PCR, high-throughput sequencing, point-of-care testing and bioinformatics has improved diagnosis of infectious diseases[13,23]. PCR involves direct sequencing of DNA which makes it more precise and allows scientists to search for multiple microbes simultaneously. It promote accurate identification of agent(s) of infection(s) improve medical care by letting doctors correctly diagnose a disease and begin treatment on time. In developed countries, remarkable progress is well documented on the use of these techniques in research, clinical trials and routine diagnosis. Most laboratories especially in developing world are not fully aware of the uses and application of molecular biology techniques[24]. In places with knowledge of these techniques they lack the expertise and capacity to handle the cost involved[25]. However, efforts are being made to extend the application of these techniques to developing countries through exchange programs, donor driven health research targeting technology transfer and capacity building.

Molecular biology and diagnosis of infectious diseases: By the time the world health proposed winning the war against infectious diseases in 1960; only about 50 novel infectious human pathogens were described[26]. When these pathogens were mention over 40 years ago, focus was on those species that could be identified easily by conventional traditional methods. This posed challenges in infectious diseases diagnosis because scientists were not able to identify non-culturable pathogens during infections due to non availability of commercial assay[27]. As such, possibility for accurate diagnosis was limited. The good news is that application of current molecular diagnostic tools has opened a new era in clinical diagnostics for previously neglected pathogensand since 1970 several emerging and re-emerging infectious pathogens are recorgnised[28,29].Molecular methods have greatly reduced difficulties encountered using traditional methods for diagnosis of infectious diseases especially those caused by bacteria species such as *Nieseriagonorrhoeae*, Intracellular pathogens – viruses, *M. genitalium,Chlamydia* sp., slow growing *M. tuberculosis* and fastidious anaerobic species,*Trichonomonasvaginalis*, because there are molecular tests kitscommercially available [30-38].

As at 2005, improved diagnostic techniques resulted in the discovery and recognition of approximately 1,400 infectious pathogens[28,29]. Recent development has focused on the production of rapid diagnostic kits based on molecular technology to accurately and rapidly detect avian flu, malaria, and dengue simultaneously. One of these techniques known as Multiplex-PCR is designed to rapidly detect multiple pathogens during infection[38]. It also helps in detecting variation in an infectious agent from its original nature either by deletions or

duplications in a large gene. Similarly, real-time multiplex PCR evaluations are capable of detecting up to 25 bacterial or fungal species in a single experiment. These techniques identify and quantify multiple pathogens of same or different species as well as their virulence genes in all clinical samples. Although the cost of carrying out some tests is challenge limiting their use to mainly in large hospitals, research and reference centers some test can also be carries out at a low cost[27].

Furthermore, diagnosis of infectious diseases is concerned with detection of the etiology of infections, pathogenetic mechanisms of chronic infections, determination of the effect of mutants on the course of the infection, monitoring of treatment progression as well as disease surveillance[30]. It is important to note that genome-based methods are relevant not only in detecting the genes of the infectious pathogens but also epidemiologically for typing of strain involved in disease outbreaks and epidemic conditions[39-41]. Spread of epidemics or hospital-acquired (nonsocomial) infections is followed and characterized with more accuracy by the identification of unique DNA fingerprints for individual pathogens[8].

The fact remains that molecular methods are specific, highly sensitive and rapid, in identifying infectious agents by the direct detection of DNA or RNA sequences unique to a particular organism. These factors contributed to the development of DNA-based specific probes capable of detecting PCR - amplified DNA of a broader variety of bacterial species, including those that cannot grow, those that are fastidious, slow in growing, or impossible-to culture[8]. This has greatly widened the scope of diagnosing and management of infectious diseases.
Furthermore, diagnosis and management of infectious diseases cut across proper healthcare delivery that is not only for the interest of infected patients but healthcare workers who may suffer cross infection at the cause of performing their duties[42,43]. Detection and quantification of genomes have potential applications in areas of occupational health practice particularly in management of occupational exposures to blood and body fluids; management of health care workers infected with hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV); and the investigation of the possible transmission of a blood borne virus either to or from a health care worker[42,43].

Immuno histochemistry, Immunoblotting, Antigen-capture enzyme-linked immunosorbent assay (ELISA) and Proteomics technique looks at the detection of protein following the principles of antigen/antibody reaction. Notable example is the direct application of the Immunomax technique in combination with the *in situ* hybridization and immunocytochemistry in illustrating tissue expression of selected DNA viruses particularly HBV and HCMV as well as antibodydetectionbyCompetitive ELISA (C-ELISA). The area of bioinformatics makes available large information on genomes of DNA analyzed deposited and easily accessible in global data bases[13]. New DNA sequencing is focused on producing catalog of human microbiome by sequencing thousands of individual organisms from different health states and correlate the microbiome to health and disease conditions[15,16,17].

Irrespective of the fact that major global health burden comes from infectious diseases, advances in the diagnosis of infectious diseases brought to light the interrelationship between infectious diseases and non infectious diseases[30]. Application of molecular diagnostic technique in medicine is extended into the use of biology of human to control non infectious conditions that are predisposed to the presence of infectious agents, such non infectious diseases like Alzheimer's Disease (AD), Neurodegenerative,

Neurobehavioral, Psychiatric, Autoimmune and Fatiguing Illnesses[31]. It gives useful reliable information on the association between viruses, bacteria, and parasitic infectious agents and cancer. So far, efforts are made in developing tools for diagnosis, new therapeutics and possibilities for vaccines[30], with special emphasis on five common types of human carcinogenic infection that is herpesviruses, retroviruses, papillomaviruses, hepatitis viruses, and *H. pylori*[30].

Bacterial infections: Molecular methods have greatly reduced difficulties encountered using traditional methods for diagnosis of infectious diseases especially those caused by bacteria species [30-36].In the 21st century Rapid diagnostic tests (RDT) technologies are molecular base[44]. Presently, laboratory automations such as GeneXpert MTB/RIF is a reckonable rapid molecular diagnostic technology for tuberculosis (TB)[45]. Point-of-care nucleic acid testing for infectious disease such in its simplest form can be used in the detection of microorganisms or specific resistance genes[45- 47]. It confirms the results of microbial cultures or even detects organisms in clinical samples[46,47].

Bacterial plasmid was the first molecular method to be used as a typing tool [48,49]. Plasmids are markers for comparing strains of for epidemiological purposes most especially in evaluating the potential spread of a bacterial species with resistant gene, presence of endemic and epidemic strains during specific period.

Fungi: Invasive fungi infections notably Aspergillosis, Athlete's Foot (Tinea Pedis), Candidiasis, Histoplasmosis, Pneumocystis Carinii Pneumonia (PCP), Sporotrichosis, and Yeast Infections have been a global problem[50- 52]. Culturing of fungi species other that *Candida* takes longer time with less sensitivity of isolation. Molecular biology techniques are promising in the analyses biological properties of fungi. Its specificity has been applied for diagnosis of bloodstream and other systemic fungi infections, which carry high morbidity and mortality rates. At present several multiples PCR primers and probes are in use as diagnostic tools[51-53].

Viral infections: Diagnosis of viral infections previously performed by serology based conventional diagnostic techniques is currently complimented by the application of molecular biology techniques in clinical and laboratory medicine. In acute infection, diagnosis is made by the detection of virus-specific IgM, rising titres of IgG or total antibody electron microscopy. However, clinical diagnosis of many viruses such as diarrhoeal and respiratory viruses remain a problem because the virus may have caused disease before the development of antibodies and in certain casessome viral infections are diagnosed by isolating the implicated virus using cell culture[54]. Limitations in using these techniques exist because although cost effective, they are time consuming, requires tasking expertise and unavoidable safety regulations.

By using molecular methods viral genomes are characterized, directly from specimens, and indirectly from isolates and in so doing providing information for epidemiological investigation, antiviral resistance, and prognostic purposes[55]. One major notable improvement is accurate diagnosis of infections like HIV using sensitive, rapid and simple DNA amplification which has contributed to decrease in rate of transmission[55].

In suspected cases, it is easy to screen participants by AutomatedRT – PCR to detect several viral infections in acute phase using serum samples. Presently, these techniques are in use for early and accurate detection of most human viruses implicated in human infections including Measles, Mumps, Herpes simplex viruses, Rota viruses Norovirus, Influenzae virus type A and B, Respiratory Syncitical virus, SARS, Dengue Japanese Encephalitis, Hepatitis B and C, West Nile, Chikungunya, HIV, Avian flu virus, and newly described H1N1 virus[56].

Parasitic infections: Malaria is a leading cause of morbidity and mortality worldwide killing over one million people each year with 90% of fatalities occurring in African children[57]. It appears molecular based techniques have assisted in overcoming the two major problems of malaria diagnosis that is sensitivity and specificity. They provide prompt diagnosis of malaria infection during emergencies[58]. Currently PCR, loop-mediated isothermal amplification (LAMP), microarray, mass spectrometry (MS), and flow cytometric (FCM) assay techniques are the most recognised accurate test methods that can identify low levels of infection not detectable by other methods[57]. Mass spectrophotometry is used to identify specific biomarker in clinical samples, it provide prompt detection of malaria infection within I min and is capable of detecting all species of *Plasmodium*. Furthermore pan-microbial oligonucleotide microarray developed for infectious disease diagnosis identifies *P. falciparum* accurately in clinical specimens[59] while flow cytometry is useful for screening in cases of clinically unsuspected malaria[57]. Automated blood cell counters (ACC) is a practical tool for malaria diagnosis that combines three principle approaches, though described for some years now, however, it is not fully available for clinical diagnosis[57,60].

Molecular methods are useful in diagnosis of Schistosomiasis a chronic and debilitating disease that affects approximately 210 million people in 76 countries around the globe and results in some 280,000 deaths per year in sub-Saharan Africa alone [61,62]. Its importance in surveillance of pattern of transmission of toxocariasis (carried by pets) and some new tickborne diseases, African trypanosomes and *T. vagilasis* infection is well documented [63].

Treatment

The growing problem of resistance has undermined the effective use of antimicrobials resulting in the loss of several lives and placing public health at serious risk. Molecular characterization has assisted in the surveillance of resistant strains and pattern of resistance. In patients with HIV-associated neurocognitive disorders, modified HIV-1 viral load assay is used for monitoring low level HIV replication in cerebral spinal. Similarly, rapid detection of

pathogens and antibiotic resistance associated with ventilator-associated pneumonia (VAP) in already in place. This has helped in guiding patients' management and accelerated progress.
Sequencing of whole genomic structures has revealed genes responsible for antibiotics resistance in clinical pathogens[64]. Not only that, sequencing the genes responsible for drug-resistance allows physicians to immediately determine which antibiotics a microbe is immune to, and this helps them choose the most effective drugs from inception of treatment.MecA possed by Methicillin-resistanct *Staphylococcus aureus* and coagulase-negative staphylococci and is associated with antibiotic resistance to Methicillin and all other B-lactam antibiotics[65]. Kyme et al,[65] recently demonstrated that high doses of the nicotinamide form of vitamin B3 in mouse stimulates a specific gene (CEBPE) capable of enhancing white blood cells' ability to combat staph infections, including methicillin-resistant *S. aureus* or MRSA within a short time. Molecular analysis revealed *Pbp1A* gene responsible for resistance to penicillin in *Streptococcus pneumonia*, SHV and TEM-β-lactamse gene sequence found in Enterobacteriaceae-producing extended-spectrum β-lactamase as well as KatG, inhA, ahpC detected in *M.tuberculosis* resistant to isoniazid and *RpoB* and rifampin respectively[66-68]. Others include thymidine kinase gene posses by Herpes simplex virus resistant to Acyclovir. Viral phosphotransferase gene in cytomegalovirus for ganciclovir resistance. Reverse transcriptase gene in HIV potentiating their resistance to Reverse transcriptase and Protease inhibitors.

Biotechnology: Clinical significance of molecular biology is extended to the area of biotechnology. Genetic modification is well known among the species of *Escherichia coli*, *Clostridium*, *Salmonella, Streptococcus*, *Listeria monocytogenes, Yersinia,* Yeasts and viruses. In medicine, this technique has been used to create strains of bacteria which are capable of producing insulin, growth hormones, components of vaccines, interferon, antigens, antibodies, antibiotics, blood clotting factors genetic markers and DNA probes for the detection of specific sequences of particular pathogens[69]. In addition to these existing products, about 369 new biotechnology medicines are evaluated experimentally for the treatment of more than 200 diseases including those caused by infectious and non infectious agents[69]. Some species of these organisms are also applied as delivery agents to target cell in gene and phage therapy[69]. As a boost to the techniques mentioned above, diagnosis and treatment of infectious diseases has improved through the use of nanotechnologies in diagnosis and vaccine development[71]. Recombinant DNA approaches are used to detect wide range of infectious agents. Although some of these findings remain research activities; others are appropriate and in use for routine diagnostic laboratory activities.

Challenges of molecular biology in diagnosis and treatment of infectious diseases: Molecular biology is promising in diagnosis of infectious conditions particularly those in which there are often few organisms present for detection by other means. The techniques used in diagnosis of infectious diseases are speedy, sensitivity and reliable. However, high level of sensitivity contributes a major challenge because PCR base diagnostic techniques can detect DNA of nonviable and viable organisms. Detection of non viable organism is an evidence of non active infection thus detecting the presence of an infectious agent may not necessary be condition of active infection. Although detection of complementary DNA by reverse- transcription PCR of messenger RNA encoded by the pathogenic organism can serve as evidence of active infection, this in turn requires clinical expertise for better interpretation of results during diagnosis.

Costs involved in procurement of reagents, apparatus personnel are a major challenge especially in developing countries. Though innovative technologies are being put in place to reduce cost of molecular diagnosis, time is necessary to actualize this.

Several biomarkers were identified for the diagnosis of several human infections; these discoveries would likely be faced with clinical trials and safety of all the possible findings and its application in the treatment of human infections[72].

Future of molecular biology: Molecular analysis of biological properties of infectious agents has brought more light into emergence and re-emergence of infectious diseases. Cost of DNA sequencing has dropped reasonably in the last few years but in most cases these technologies are not yet available for routine medical care. Reduction in cost, and the use of molecular diagnostic that are speedy, accurate, and user friendly will ensure further increase in the role of molecular methods in diagnosis and treatment of infectious conditions.New automated quantitative multiplex platforms are being developed for the diagnosis and appropriate management of infectious diseases

Detection of viral bacterial, parasitic and fungal organisms provides a challenge to the clinical diagnosis, because clinicians and laboratorians

require clinical expertise to relate the presence of molecular fragments of each organism to the presence of infection. Putting this in place in the nearest future would greatly enhance the diagnosis and treatment of infectious diseases.

Molecular biology techniques are used to detect resistant genes in infectious agents however, phenotypic methods are needed to detect emerging resistance. The presence of a resistant gene may not denote resistance because such gene may not have been expressed. In future, there would be more enhanced point-of-care testing using nucleic acid techniques that are cost-effective, robust, and user-friendly through rapid PCR using microfluidic technology and LAMP.

Conclusion

Molecular biology techniques are used to improve sensitivity and speed of diagnosis in infectious diseases and treatment. Several techniques has been described, however, the urgency of diagnosis, the experience of the physician, the effectiveness of healthcare workers, and budget resources greatly influence the choice of diagnostic method. Utilizing molecular biology based approach in diagnosis and treatment of infectious diseases is a step towards achieving specific and detailed identification of infectious agents, accurate and specific diagnosis, monitoring, broader epidemiological surveillance and proper diseases and patient's management.

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Nigerian Journal of Clinical & Biomedical Research (Jun. 2012) Vol. 6 No.1 28 – 32

Review Article

Prevention of Mother-to-Child Transmission of Hepatitis B virus

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Summary

Perinatal transmission accounts for the majority of chronic Hepatitis B virus (HBV) infections in regions with high prevalence (i.e. >7%). Nigeria is a hyper-endemic region for HBV infection; therefore prevention of mother-to-child-transmission (PMTCT) is essential. Maternal screening and neonatal immunoprophylaxis (active and passive) have greatly reduced vertical transmission in countries where such programmes have been established. Antenatal immunoglobulin and antiviral therapy in the third trimester of pregnancy in high-risk women with chronic HBV infection may also decrease the risk of perinatal transmission. Mode of delivery and breastfeeding has not been proven to significantly increase the risk of vertical transmission. A comprehensive discussion of the risks and benefits of therapy with the patient is necessary, in addition to the involvement of the hepatologist and paediatrician for follow-up. Prophylaxis however remains the best method of prevention of perinatal transmission.

Keywords: Hepatitis, perinatal, transmission

Introduction

Hepatitis B infection, one of the major infectious diseases of the liver is caused by a small enveloped double-stranded DNA virus, the hepatitis B virus (HBV) belonging to the *Hepadnaviridae* family. Baruch Blumberg and colleagues first described HBV as the "Australia antigen" in 1965[1], but this was later named hepatitis B surface antigen (HBsAg) in patient blood. Hepatitis B e antigen (HBeAg) has also been recognized as a marker for high infectivity of the disease.

It is estimated that more than two billion people have been infected by HBV world wide and 350 million people have chronic infection, resulting in 2 million deaths annually.[2] In areas of high prevalence, acquisition of the infection is mainly through perinatal (vertical) transmission and early childhood (horizontal) infection.[3] Nigeria is one of the highly endemic countries for viral hepatitis and currently, about 18 million Nigerians are infected.[4] Thus, there is a significant potential burden of perinatally -acquired HBV infection. About 10 - 20% of HBsAg women transmit the virus to their neonates, while women who are seropositive for both HBsAg and HBeAg have vertical transmission of up to 90%.[5] The highest risk of chronic infection has been found among infected neonates born to HBeAg positive carrier mothers, as well as children infected before 6 years of age.[6]

Perinatal transmission of HBV infection has declined significantly in developed countries such as the United States, due to the successful implementation of universal screening of pregnant women and vaccination programmes.[7]However, many high-prevalence

countries lack adequate vaccination coverage with a consequently higher risk of vertical transmission. Hepatitis B vaccine coverage in Nigeria is still low at 41%, though it has increased over the last few years.[8]

The prevalence of HBsAg in normal population in Nigeria ranges from 2.7 to 13.3%.[9,10] Among pregnant women in Nigeria, HBsAg prevalence varies across regions, with higher prevalence rates of 8.3% and 11.6% reported in Zaria and Maiduguri respectively[11,12], while lower prevalence reports include 2.89% in Enugu[13] and 4.3% in Port Harcourt.[14]

Few studies have been done in Nigeria on the perinatal transmission of Hepatitis B virus. Onakewhor and colleagues[15] in Benin City reported a perinatal transmission rate of 42·86% using HBsAg immunoassay, while a recent study done in Ile-Ife revealed a perinatal transmission rate of 72% using HBV DNA assay.[16]

The effects of chronic HBV infection on pregnancy outcomes have not been clearly defined. Though HBV infection does not appear to cause birth defects, a higher incidence of low birth weight among infants born to mothers with acute infection during pregnancy has been observed. However, one large study demonstrated no differences in gestational age at delivery, birth weight, incidence of prematurity, neonatal jaundice, congenital anomalies or perinatal mortality comparing HBsAg-positive women with controls.[17]

Detection of perinatal HBV infection: Different kinds of fetal samples have been used to assess perinatal HBV infection, such as amniotic fluid, cord blood, and placental tissue collected intra- or post-partum. This method of detecting fetal infection is non-invasive, economical and is done in most studies.[18,19] Since postnatal samples were inevitably contaminated by maternal blood during labor, some authors have preferred using neonatal peripheral blood, which was collected prior to postnatal immunization, to detect intrauterine infection. This method was usually achieved by neonatal femoral venous puncture and might better reflect the intrauterine status.[20] However, this method cannot identify the time of infection (before or during labor) because maternal blood leakage into fetal circulation is unavoidable during labor.[21] Use of fetal samples obtained from invasive prenatal diagnosis such as amniocentesis and PUBS to evaluate the fetal intrauterine status has also been done in some studies.[21] Though this seems to be an improved approach for extraction of purified fetal samples, these procedures are invasive and require a high level of technical skill.

The detection rate of intra-uterine HBV infection is higher with HBV DNA assay than the HBsAg assay.[16,22] This difference is due to a greater sensitivity of PCR method compared to rapid immune-chromatography method. The HBV DNA shows directly the condition of replication and infectivity of the virus in vivo, and this can be reflected through HBV DNA quantitation using the polymerase chain reaction (FQ-PCR) test clinically.[23] A reason postulated for the increased level of HBV DNA expression in neonatal blood is that the incubative virus replicates in bulk with fetal liver maturation during later gestation.[21]

Risk factors for vertical transmission of HBV: Vertical transmission may occur in the antenatal, intrapartum periods and rarely, in the postnatal period. Antenatally, this risk may be amplified by invasive prenatal diagnostic procedures, such as amniocentesis and cordocentesis. Although the risk of fetal hepatitis B infection through amniocentesis is low[19], the use of noninvasive methods of prenatal risk screening such as nuchal translucency, triple screening, and anomaly ultrasound scanning, may obviate the need for invasive diagnostic procedures. For women who still require amniocentesis, every effort should be made to avoid inserting the needle through the placenta.[19]

Most perinatal transmission is believed to occur at or near the time of birth, because neonatal vaccination prevents newborn infection in about 80–95% of cases.[24] During delivery, contact of the neonate with cervical secretions and maternal blood is believed to be a contributing factor to perinatal transmission of HBV. Risk factors for transplacental transmission of HBV include maternal HBeAg positivity, HBsAg titre and HBV DNA level.[24]

HBeAg is a seromarker related to infective HBV particles and its seropositivity might represent a high level of viral replication in hepatocytes. In the absence of immune-prophylaxis, the risk of transmission from HBsAg/HBeAg-positive mothers to their fetuses is almost 90%.[5]

Studies have shown that the level of HBV DNA in maternal serum during the antenatal period is the most important predictor of chronic infection in the newborn.[16,25] The development of persistent infection is said to be directly related to the quantity of DNA to which the infant was exposed; HBV DNA levels of $>10^8$ copies/ml are associated with a greater risk of transmission.[23,26]

With HIV/AIDS ravaging most parts of sub-Saharan Africa, co-infection with hepatitis B with which it is closely linked is a rapidly growing

public health concern. This is because the hepatitis virus and HIV share a common mode of transmission, which is the sexual route. A recent study on HBsAg prevalence among patients with HIV in Gombe showed a prevalence of 26.5%.[27] In an American series, HIV-infected obstetric patients with HBV co-infection were reported to have lower CD4 counts when compared with women with both HIV and HCV or those with HIV alone. The women with chronic HBV also had lower median CD4 counts than those who had cleared previous HBV infection.[28] It is however not clear whether HBV co-infection conferred additional immune suppression in this group.

Strategies for the prevention of mother to child transmission of HBV: Routine screening of pregnant women for HBsAg is an important initial step in the prevention of vertical transmission of HBV. Women who are detected to be HBsAg positive can then be evaluated and managed or referred to a higher facility as appropriate. This strategy has helped in the reduction of perinatal HBV infection in many developed countries. In Nigeria, screening of antenatal women for hepatitis B virus is not a routine practice in many health facilities. Furthermore, routine vaccination of newborns is not widely available in low resource settings within this environment. Even though Nigeria approved the inclusion of hepatitis B vaccine in its National Program on Immunization (NPI) in 1995, the vaccine only became widely available in 2004.[29] Despite the availability of the vaccine through the NPI, immunization coverage for Hepatitis B and other childhood vaccines is still suboptimal, especially in the rural areas.

With the 'e'-antigen being a risk factor for transmission, HBeAg screening in HBsAg positive pregnant women can further help in identifying those who are at possibly higher risk of vertical transmission, so as to implement measures for PMTCT. The serum/plasma HBV DNA assay is useful for both initial assessment and monitoring, as it is a reliable marker of active HBV replicationand high levels are associated with a greater risk of transmission.[23]

Maternal Hepatitis B Immunoglobulin (HBIG) administration during pregnancy has been employed in PMTCT in many developed countries. Shi et al[30] in 2010 carried out a systematic review of 37 randomised controlled trials (RCTs) to evaluate the efficacy and safety of using hepatitis B immunoglobulin during pregnancy to prevent vertical transmission of HBV. A total of 5,900 newborns of asymptomatic HBsAg positive mothers were included. Compared with the control group, newborns in the HBIG group had a lower intrauterine infection rate (indicated by HBsAg and/or HBV DNA) and a higher protection rate (indicated by anti-HBs). A similar trend was found in MTCT by 9–12 months after birth, using the same parameters.[30]

The use of antiretrovirals such as lamivudine (FDA pregnancy category C), and more recently, tenofovir (Category B) in PMTCT of HBV has also been employed. This therapy is usually commenced in the third trimester and combined with neonatal vaccination. A systematic review and meta analysis of RCTs evaluating the efficacy of lamivudine in reducing in -utero transmission of HBV revealed that lamivudine administration in HBV carrier–mothers with high risk of infectivity in late pregnancy was associated with a lower incidence of intra-uterine infection and MTCT at 9-12months. [31]

Although caesarean delivery has been proposed as a means of reducing mother to child transmission (MTCT) of HBV, where the immunoprophylaxis is given, the mode of delivery does not appear to have a significant effect on perinatal transmission.[32] Delivery by caesarean section for the purpose of reducing MTCT of HBV is not presently recommended by either the Centers for Disease Control (CDC) or the American College of Obstetrics and Gynaecology (ACOG).[2,33]

Breastfeeding has been suggested as an additional mechanism by which infants may acquire HBV infection, because small amounts of Hepatitis B surface antigen (HBsAg) have been detected in some samples of breast milk. However, several studies have reported that breastfeeding carries no additional risk that might lead to vertical transmission.[34] Although concerns have been raised that breast pathology such as cracked or bleeding nipples could expose the infant to infectious doses of HBV**,** with appropriate hepatitis B immunoprophylaxis, breast-feeding poses no additional risk for transmission from infected hepatitis B virus carriers.[34] Furthermore, the American Academy of Pediatrics recommends that HBV infection should not be considered a contraindication to breastfeeding of infants who receive the approved hepatitis B immune globulin (HBIG) and HBV vaccine.[35]

Perinatal transmission of HBV can be prevented by HBV vaccination in 75-80% of cases if given within 24 hours of birth. By co-administering passive–active immuno-prophylaxis [HBV vaccine + HBIG], transmission rates can be further reduced to between 0% and 14%.[36] This finding was corroborated by the systematic review and meta-analysis of 26 RCTs carried out by Lee et al[37]

on the effect of HBV immunisation in newborns of HBsAg positive mothers. It was observed that HBV vaccine and HBIG administered singly or in combination prevented hepatitis B occurrence in such newborn infants.[37]Nevertheless, the most important determinant of prophylaxis failure has been shown to be maternal serum HBV DNA levels (viral load). Transmission rates as high as 32%, despite immunoprophylaxis, have been reported in infants born to mothers with HBV DNA concentrations >10^9 copies/mL.[38]

The management of HBV infection during pregnancy requires a serological assessment for HBsAg, HBeAg, HBcAb and HBsAb. A history of perinatal transmission and an assay of viral load at 28weeks will guide further management decisions. All children of HBsAg-positive mothers should receive Hepatitis B Immunoglobulin in addition to vaccination at birth. Women with high viral loads can be considered for treatment with antiviral therapy in the 3rd trimester, but a comprehensive discussion of risks and benefits needs to take place before opting for treatment. The HBV DNA threshold for initiating treatment depends on a history of perinatal transmission. If a previous child was HBV positive, the threshold for treatment may be lower (HBV DNA > 10^6copies/mL). However, if the previous child was not HBV positive, treatment might be considered with HBV DNA levels >10^8 copies/mL.[7]

Conclusion

The high prevalence of hepatitis B infection in this environment, and among pregnant women in particular, calls for concern. The various modalities of preventing MTCT should be adapted to the patient and available facilities. The benefits of treatment appear to be most pronounced in cases with high maternal viremia. The involvement of the physician (hepatologist) and paediatrician in the management of infected individuals is essential. The development of a national protocol for PMTCT is vital and further research into the most appropriate as well as cost effective interventions for PMTCT of Hepatitis B virus in our low-resource setting is required.

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Research Article

Efficacy of a combination long lasting insecticidal net (PermaNet® 3.0) against pyrethroid resistant *Anopheles gambiae s.s* and *Culex quinquefasciatus*: an experimental hut trial in Nigeria

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Summary

PermaNet® 3.0: a mosaic combination long-lasting insecticidal net (LLIN) combining deltamethrin coated polyester side panels and a deltamethrin with PBO incorporated polyethelene roof was designed to give increased efficacy against pyrethroid-resistant malaria vectors. Evidence supporting the efficacy of this product is mainly limited to comparison with PermaNet® 2.0: a product relying on deltamethrin alone. Here we report on performance of PermaNet® 3.0 on free flying *Anopheles gambiae* and *Culex quinquefasciatus* in comparison to permethrin-impregnated Olyset® nets in an area with multiple pyrethroid resistance mechanisms. Prior to the field test, net samples were washed using standard WHOPES washing protocols and bio-efficacy conducted with the reference susceptible Kisumu strain of *A. gambiae*. Additional bioassay tests were conducted on the net samples with a resistant strains of *A. gambiae s.s* and *C. quinquefasciatus* prior to and after the field trial. Field efficacy was expressed in terms of deterrence in hut entry, blood-feeding inhibition, induced exophily and mortality.Laboratory cone bioassays prior to and after field test showed high mortality of *A. gambiae s.s.* (>70%)in PermaNet® 3.0 which was consistent with the field data. Increased bio-efficacy likely due to the synergist effect of PBO and deltamethrin was obvious from *in situ* bioassays, with significant mortality of resistant *A. gambiae s.s.* recorded following exposure to the roof panel. Experimenal field data showed that PermaNet® 3.0 induced a level of deterrence and exophily against *A. gambiae* and *C. quinquefasciatus* similar to that of the Olyset® net, but the feeding rate of *A. gambiae s.s*. in PermaNet® 3.0 was highly minimized compared to the Olyset® net. In spite the presence of both *kdr* and MFO resistance mechanisms, the proportion of *A. gambiae s.s* and *C. quinquefasciatus* killed by PermaNet® 3.0 was significantly higher than the Olyset® net ($P < 0.01$) confirming the increased bioefficacy of PermaNet® 3.0.PermaNet® 3.0 outperformed the Olyset® net and could provided additional protection in terms of reduction in blood feeding and increase in mosquito mortality: a plausible tool against pyrethroid resistant mosquitoes.

Key words: long-lasting nets, PermaNet®, P.B.O, *Anopheles gambiae , Culex quinquefasciates, kdr,*

Introduction

The development of long-lasting insecticidal net technology has been described as a breakthrough in malaria prevention[1]. However, there is increasing evidence from West[2,3,4] Central[5,6] and East Africa[7,8] on the reduced efficacy of insecticide treated nets (ITNs) and long lasting insecticidal nets (LLINs) in areas with high levels of mosquito resistance to pyrethroid. Although alternative insecticides on nets samples have been tested against pyrethroid resistant *Anopheles*[9,10], none is as effective as pyrethroid in terms of the excito-repellency properties and fast knock down action of pyrethroid which are major features that provide protection against mosquito bites.

Two major mechanisms play an important role in *Anopheles* resistance to insecticides: target site insensitivity and metabolic enzyme-based resistance[11,12]. Target site insensitivity to

pyrethroid is associated with a single point mutation commonly referred to as knock down resistance (*kdr*) leading to modification of the voltage-gated sodium channel gene making it less susceptible to the binding of pyrethroid[13] . Metabolic-based resistance mechanism is principally associated with mixed function oxidases (MFO) [14] . Evidence of both resistance mechanisms had been reported in the malaria mosquito: *Anopheles gambiae s.s* from Nigeria[15,16]. Insecticide resistance management strategies include, rotational use of insecticide, insecticide mixtures and insecticide synergism. Insecticide synergism in particular is used to enhance the potency of commercial aerosols[17].

With the threat of insecticide resistance, newer methods of preserving the efficacy of available public health insecticides need to be put in place. PermaNet® 3.0, a new LLIN, was designed to give increased efficacy against pyrethroid-resistant malaria vectors. This mosaic LLIN combines deltamethrin coated polyester side panels and deltamethrin with the synergist piperonyl butoxide (PBO) incorporated in the polyethylene roof. PBO is an inhibitor of MFO with potential to reduce activity of enzymes associated with this resistance mechanism. The principle behind the development of PermaNet® 3.0 is based on the notion that a combination of a pyrethroid and PBO will also enhance the rate of insecticide penetration in the insect and increase the efficacy of the net. The World Health Organization Pesticide Evaluation Scheme (WHOPES) is yet to set criteria for evaluating products that have an effect on insecticide resistant vectors. A practical approach is to compare the efficacy of PermaNet®3.0 with LLINs that have received full WHOPES recommendation. At present, only PermaNet® 2.0 and Olyset® nets have met these criteria[18-20] with Yorkool® nets given a full recommendation but based only on equivalence with PermaNet® 2.0. Aside from a parallel study in Benin[21], previous field evaluations of PermaNet® 3.0 were made in comparison with PermaNet® 2.0, a product from the same manufacturer but relying on deltamethrin alone[22,23]. Following a review of the available evidence on the efficacy of PermaNet® 3.0[24], an interim recommendation was granted by WHOPES based on the need for additional proof of evidence as a requirement for developing full recommendations on the use of the product.

The current study was undertaken to compare the performance of PermaNet® 3.0 with permethrin-incorporated Olyset® nets in experimental huts in north-central Nigeria.

Materials and method

Study site and experimental huts: The study was conducted in experimental huts situated at the Nigerian Institute of Medical Research field station at New Bussa (9^{o} 53'N; 4^{o} 31' E). The malaria mosquito *Anopheles gambiae s.s.* in the area exhibits a high level of pyrethroid resistance associated with both knock down resistance (*kdr*) and metabolic-based resistance with mixed function oxidases (MFO) (Awolola unpublished). Six experimental huts were built on a concrete floor following the pattern of huts used in West Africa[25]. The styles of the huts simulates domestic habitations and were purposely built with the front side facing perennial mosquito breeding sites.

Insecticide susceptibility test: Prior to the commencement of the experimental hut evaluation, *Anopheles* and *Culex* larvae were collected around the field site and reared to adulthood. Insecticide susceptibility test was conducted with permethrin (0.75%) and deltamethrin (0.05%) on 2-3 day old non-blood fed female mosquito using WHO test kits[26]. Mosquitoes tested were identified morphologically and specimens belonging to the *A. gambiae s.l.* further analysed by PCR[27]. A population *of Anopheles gambiae* that survived the insecticide exposure was divided into two: a subset was analysed for the presence of the *kdr* mutation[13]. The second subset was induced to lay eggs in the laboratory insectaries, and the F1 progeny used for synergist and biochemical analysis using the protocol described in our previous study[16] with reference to the Kisumu susceptible strain of *A. gambiae* .

Bioassay on net samples: Before field test, net samples: PermaNet® 3.0, Olyset® and conventionally treated polyester net (CTN) samples were washed according to the WHOPES phase 1 protocol[25]. Because of the limited information on insecticide regeneration on Olyset®, incubation of the Olyset® net samples was adapted from a previous phase 1 study in our laboratoryand bio-efficacy evaluations conducted with the Kisumu susceptible reference strain of *A. gambiae s.s.* Separate bioassay tests were conducted with resistant strains of *A. gambiae s.s* and *C. quinquefasciatus* prior to and after the field test.

Experimental hut evaluation

The experimental set up was made of six treatment arms:

1. Untreated net
2. PermaNet® 3.0 unwashed

3. Olyset® unwashed
4. PermaNet® 3.0 washed 20 times
5. Olyset® washed 20 times
6. Polyester net conventionally treated (by deltamethrin at 25 mg/m^2) and washed until just before exhaustion (<80% mortality in cone bioassay or <95% knockdown after 1h).

Each treatment arm consisted of 7 whole nets, 6 of which were used in the experimental huts. The seventh net of each arm was used as a reference sample and preserved for chemical analysis. Nets were purposely holed with 6 holes of 4 x 4 cm cut in each net. The conventionally treated net (CTN) washed until just before exhaustion (CTN) was used as the positive control. The 12 weeks Latin Square design was adapted from WHO guidelines for phase 2 field trials[25]. Each treatment arm was rotated each week among the huts, with rotation of adult male volunteers who slept under the nets each night. Field efficacy was expressed in terms of mosquito deterrence in hut entry, blood-feeding inhibition, induced exophily and mortality.

Chemical analysis: The 7th net of each treatment arm was not tested in the huts but instead stored at ambient temperature and processed for chemical assays together with samples used in the field. Chemical assays were carried out according to CIPAC method at an independent laboratory (TUV SUD PSB Pte Ltd, Singapore: test report reference no. 719186073-CHM10/02-CSY &719186073-CHM10-JS-CR01).

Statistical analysis

Because of the variation in the number of mosquitoes collected over different nights, the number of mosquitoes entering the huts was analysed using non-parametric Kruskall-wallis tests. The proportion of mosquitoes that exited into the traps, the proportion killed within the hut and the proportion that was blood fed in each experimental arm were analysed using logistic regression (STATA 6 Software).

Results

Mosquito resistance status

The *Anopheles* population for the resistance test consisted of 61.6% *A. gambiae s.s* and 38.4% *A. arabiensis. Anopheles gambiaes.s* was resistant to permethrin and deltamethrin with an average mortality rate of 75.7 and 79.5% for permethrin and deltamethrin respectively. *Anopheles arabiensis* was susceptible to both insecticides (Table 1). The mortality rate for *Culex quinquefasciatus* was 61.3 and 74.1% in permethrin and deltamethrin respectively (Table 1).

The *kdr* frequency was 45% in the resistant *A. gambiae* population. The difference in mortality 24 h post-exposure between synergized and unsynergized field population of *A. gambiae* exposed to permethrin was highly significant ($P < 0.001$). Synergized and unsynergized samples exposed to deltamethrin gave similar results (Table 1). Biochemical analysis revealed a

Table 1: Susceptibility status of *Anopheles gambiae s.s, A. arabiensis, Culex quinquefasciatus* and synergist test comparing pyperonyl butoxide synergized and unsynergized resistant population of *A. gambiae s.s* collected at the study site

Mosquito species	No. exposed (24 h % mortality)[a]			
	Permethrin (0.75%)	4% PBO + 0.75% permethrin	Deltamethrin (0.05%	4% PBO + 0.05% deltamethrin
Anopheles gambiae s.s.	140 (75.7)	120 (88.8)	132(79.5)	120 (94.5)
Anopheles arabiensis	102 (100)	ND	118(100)	ND
Culex quinquefasciatus	320 (61.3)	ND	310 (74.1)	ND

PBO: Piperonyl Butoxide. ND: not determined

[a] Figures in parentheses denote % mortality of the mosquitoes exposed

significantly increased level of monooxygenase in the resistant *A. gambiae*population compared with the reference susceptible Kisumu strain(P= 0.027).

Laboratory bio-efficacy of net samples: The unwashed PermaNet® 3.0 and the Olyset® net samples produced 100% mortality against the susceptible Kisumu
reference strain of *A. gambiaes.s* and remained effective after 20 washes. The CTN samples declined with successive washes and after 5 washes efficacy had decreased from 100% at baseline to 80%.
Five washes was therefore selected as the number of washes required before CTN

exhaustion. Bioassay tests of washed and unwashed net samples with the resistant mosquitoes population prior to the field test revealed high mortality of *A. gambiaes.s* (70-89%) and *C.quinquefasciatus* (60-78%) only in PermaNet® 3.0 (Figure 1).
There was a significant increase in mortality of *A. gambiaes.s* (85- 96%) and *C. quinquefasciatus*(74-89 %)in the roof panel of PermaNet®3.0 compared to the side panels. This however declined with a margin of 7% with *C. quinquefasciatus*after 20 washes.

Efficacy of treatments in experimental huts

Vector population: The 432 hut-night collections (72 nights x 6 huts) produced 2306 female *Anophelesgambiaes.l*(62.2 % *A. gambiaes*.s.; 37.8% *A. arabiensis*) and 4873*C.quinquefasciatus*. The overall mean number of female mosquitoes collected per hut per day was 5.3% *A.gambiaes.l* and 11.3 *C.quinquefasciatus.*The final results for the field efficacy (Table 1 and 2) is presented for *A. gambiaes.s* and *C. quinquefasciatus*were resistant to deltamethrin and permethrin.

After 12 weeks use in the experimental hut, both PermaNet® 3.0 and the Olyset® net still showed high efficacy (>98%) against the susceptible Kisumu strain of *A. gambiaes.s*.There was however, a significant decline in the bio-efficacy of the Olyset® relative to PermaNet® 3.0 when tested on the resistant strain of *A. gambiae*and *C. quinquefasciatus.*

Deterrence in hut entry: The number of mosquitoes in the huts with the untreated net was higher than with any of the other treatment arms. The treatment arms with insecticide deterred more *Anopheles* than *Culex.*There was >13% reduction in huts entry of *Anopheles* in each of the treatments when compared with the huts with the untreated net. Although there was an increase in the deterrence of *A. gambiae* in huts with PermaNet® 3.0 compared to Olyset®, the difference was not statistically significant. The percentage deterrency of *C.quinquefasciatus*was also similar for both PermaNet® 3.0 and Olyset® (Table 3).

Figure 1: Bio-efficacy of PermaNet® 3.0, Olyset® and CTN against resistant field population of *Anopheles gambiae s.s* and *Culex quinquefasciatus* prior to field use.

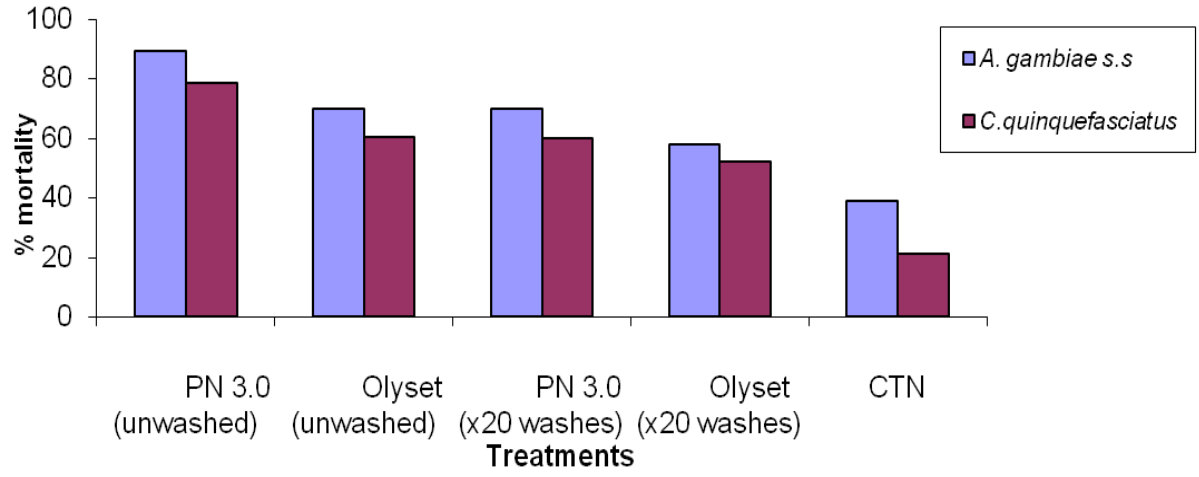


Table 2: Summary of experimental hut trial for *Anopheles gambiae s.s.*

Outcome Measures	Control untreated net	PermaNet 3.0 (unwashed)	Olyset (unwashed)	PermaNet 3.0 (x20 washes)	Olyset (x20 washes)	CTN (x5 washes)
ENTRY RATE						
Total females Caught	470	344	368	356	376	392
Females caught Per night	6.5[a]	4.8[b]	5.1[ab]	4.9[a]	5.2[ab]	5.4[ab]
% deterrence (Tc-Tt)/Tc x 100	-	26.8	21.7	24.2	20.0	16.5
EXIT RATE						
Total females in traps*	110	142	162	139	161	132
% exophily (95% CI)	23.4[a] (20.2-25.8)	41.3[b] (39.6-44.4)	44.0[b] (43.1-50.4)	39.0[b] (37.8-43.9)	42.8[b] (40.9-45.1)	33.7[ab] (30.8-37.9)
BLOOD FEEDING RATE						
Total females blood fed	270	10	47	16	68	108
% blood fed (95% CI)	57.5[a] (54.8-59.1)	2.9[b]	12.8[c] (10.2-13.8)	4.5[b]	18.1[c] (16.5-21.2)	27.5[d] (24.5-30.1)
% blood feeding inhibition (%BFc-%BFt)/BFcx 100	-	94.9	77.7	92.1	68.5	52.2
% personal protection (BFc-BFt)/ (BFc x 100)	-	96.2[a]	82.6[b]	94.1[a]	74.8[b]	60.0[c]
MORTALITY RATE						
Total females dead	20	252	144	246	104	70
% overall mortality (95% CI)	4.3[a] -	73.3[b] (70.6-75.9)	39.1[c] (37.1-41.8)	69.1[b] (64.6-72.9)	27.6[d] (24.1-29.2)	17.9[e] (15.8-19.9)
Overall insecticidal effect (Dt-DC/Tc x 100)	-	49.4[a]	26.4[b]	48.1[a]	17.9[c]	10.6[d]

* Number on the same row with similar superscript do not differ significantly (P >0.05).

Tc = Total number of female *Anopheles gambiae s.s* collected in the control arm (non-treated net)

Tt = Total number of female *Anopheles gambiae s.s* collected in each treated arm

BFc = Total number of blood fed female *Anopheles gambiae s.s* collected in the control arm

BFt = Total number of blood fed female *Anopheles gambiae s.s* collected in the treated arm

Dc = Total number of dead female *Anopheles gambiae s.s* in the control arm

Dt = Total number of dead female *Anopheles gambiae s.s* in the treated arm

Table 3 Summary of experimental hut trial for *Culex quinquefasciatus*

Outcome Measures	Control untreated net	PermaNet 3.0 (unwashed)	Olyset (unwashed)	PermaNet 3.0 (x20 washes)	Olyset (x20 washes)	CTN (x5 washes)
ENTRY RATE						
Total females Caught	990	748	780	762	788	805
Females caught Per night	13.8[a]	10.4[b]	10.8[b]	10.6[b]	10.9[b]	11.2[b]
% deterrence (Tc-Tt)/Tc x 100	-	24.4	21.2	23.0	20.4	18.9
EXIT RATE						
Total females in traps	182	272	255	242	234	194
% exophily	18.3[a]	36.4[b]	32.6[b]	31.8[b]	29.7[b]	24.1[c]
(95% CI)	(16.9-20.1)	(32.5-39.2)	(29.2-35.6)	(28.6-34.9)	(25.8-33.4)	(21.2-28.8)
BLOOD FEEDING RATE						
Total females blood fed	620	72	197	88	251	307
% blood fed	62.7[a]	9.6[b]	25.2[c]	11.5[b]	31.9[cd]	38.1[d]
(95% CI)	(57.9-65.1)	(6.8-11.2)	(20.4-27.9)	(8.2-13.5)	(28.6-35.6)	(33.1-42.4)
% blood feeding inhibition (%BFc-%BFt)/BFcx 100)	-	84.6	59.8	81.6	49.2	39.2
% personal protection	-	88.3[a]	68.2[b]	85.8[a]	59.5[bc]	51.9[c]
MORTALITY RATE						
Total females dead	28	487	164	315	113	118
% overall mortality	2.8[a]	65.1[b]	21.0[c]	41.3[d]	14.3[e]	14.6[e]
(95% CI)	-	(60.2-69.6)	(16.2-25.8)	(37.8-46.2)	(10.9-16.3)	(11.2-16.9)
Overall insecticidal effect (Dt-DC/Tc x 100)	-	46.4[a]	13.7[b]	28.9[c]	8.9[d]	9.0[d]

Induced exophily: All treatment arms increased the proportion of *A. gambiae* and *C. quinquefasciatus* in the veranda and exit traps compared to the untreated control. Both PermaNet® 3.0 and Olyset® nets induced greater exophily against resistant *A. gambiaes.s*than *C. quinquefasciatus* . The extent of induced exophily in PermaNet® 3.0 ranged from 32 to 41 % for *A. gambiae* and 23 to 25% for *C. quinquefasciatus*. There was no significant increase in the exit rate of *A. gambiae*and *C. quinquefasciatus* in huts with PermaNet® 3.0 and Olyset®.

Blood feeding: Both *A. gambiae* and *C. quinquefasciatus* showed a high rate of blood feeding (>57%) in the hut with the untreated net (Figure 2) but *C. quinquefasciatus* showed a significantly higher feeding rate than *A. gambiae* in all treatment arms with insecticide, resulting in lower blood feeding inhibition of the former (Table 3). There was >92 % blood feeding inhibition of *Anopheles* in huts with PermaNet® 3.0 (Table 2). In contrast to the Olyset® net, the blood feeding inhibition of *A.gambiae*and *C. quinquefasciatus*in huts with unwashed PermaNet® 3.0 did not differ from the huts with the washed nets (Table 2 and 3).

Mortality: PermaNet® 3.0 induced significantly greater mortality against resistant A. gambiaes s. than C. *quinquefasciatus*(Figure 3).The unwashed PermaNet® 3.0 produced the highest mortality rates:73.5 and 65.1% for *A. gambiae*and *C. quinquefasciatus* respectively. PermaNet® 3.0 had significantly higher mortality for both *A. gambiae*and *C. quinquefasciatus* when compared to the Olyset® nets (P<0.01). Washing PermaNet® 3.0 up to 20 times did not significantly reduced the mortality of *A. gambiae*but mortality declined with *C. quinquefasciatus*after 20 washes (P<0.01). Washed Olyset® significantly reduced the mortality of both *A. gambiae s.s.* and *C. quinquefasciatus*.

Figure 2: Proportion of blood fed Anopheles gambiae s.s. and Culexquinquefasciatus in experimental huts with PermaNet® 3.0,Olyset® net and CTN.

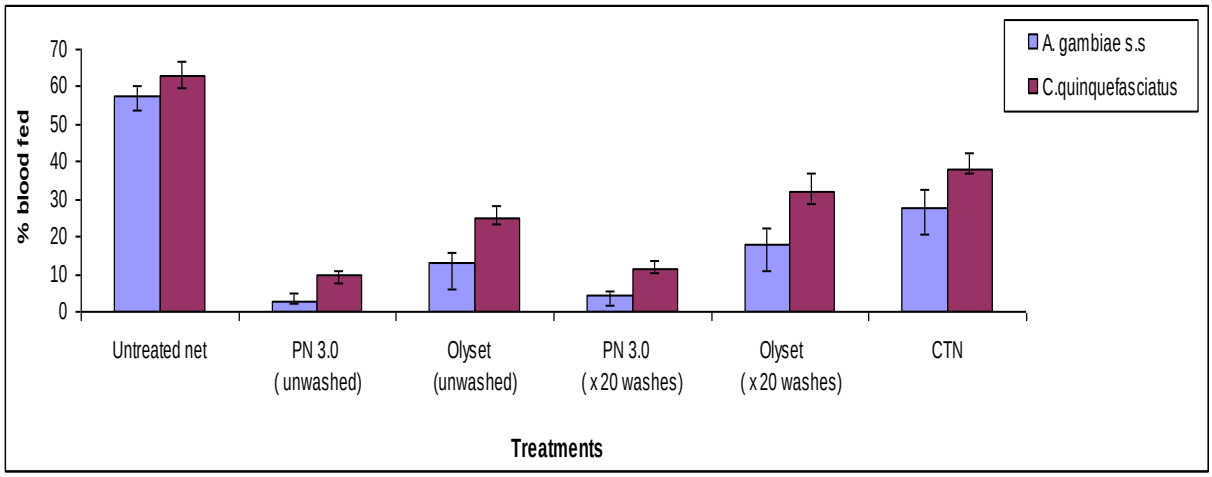


Figure 3: Mortality rate of *Anophelesgambiaes.s*. and *Culexquinquefasciatus* in experimental huts with PermaNet® 3.0,Olyset® net and CTN

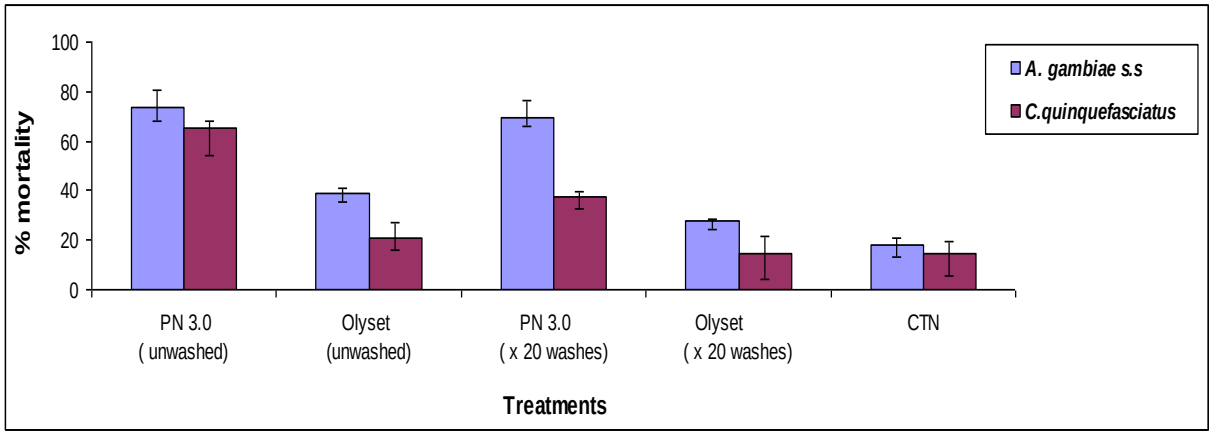


Figure 4: (A) Chemical analysis of deltamethrin in side panel of PermaNet® 3.0 and (B) permethrin in Olyset® nets used in the experimental hut tria

Fig 4A

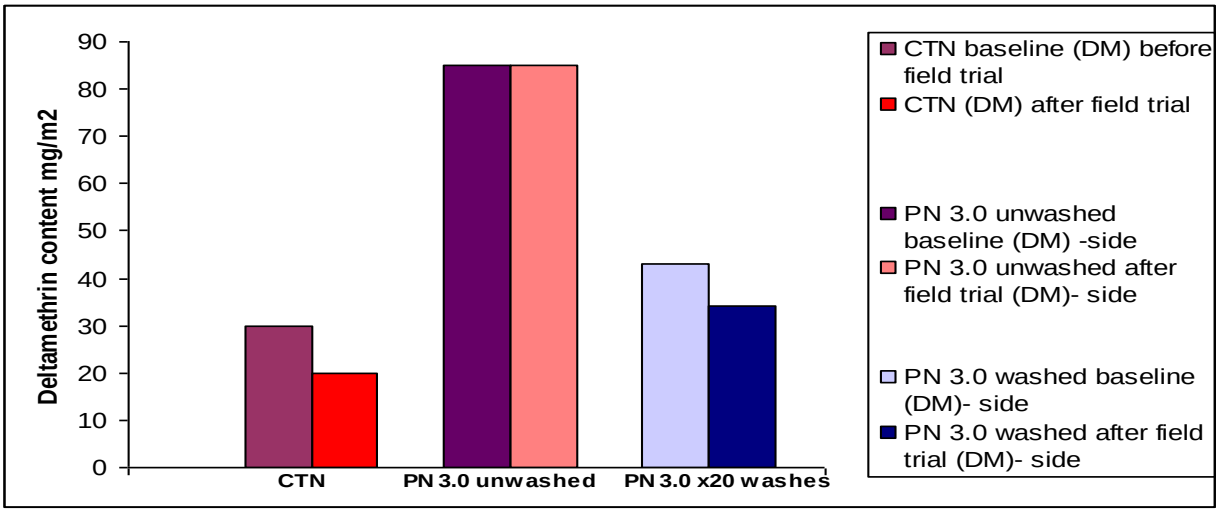
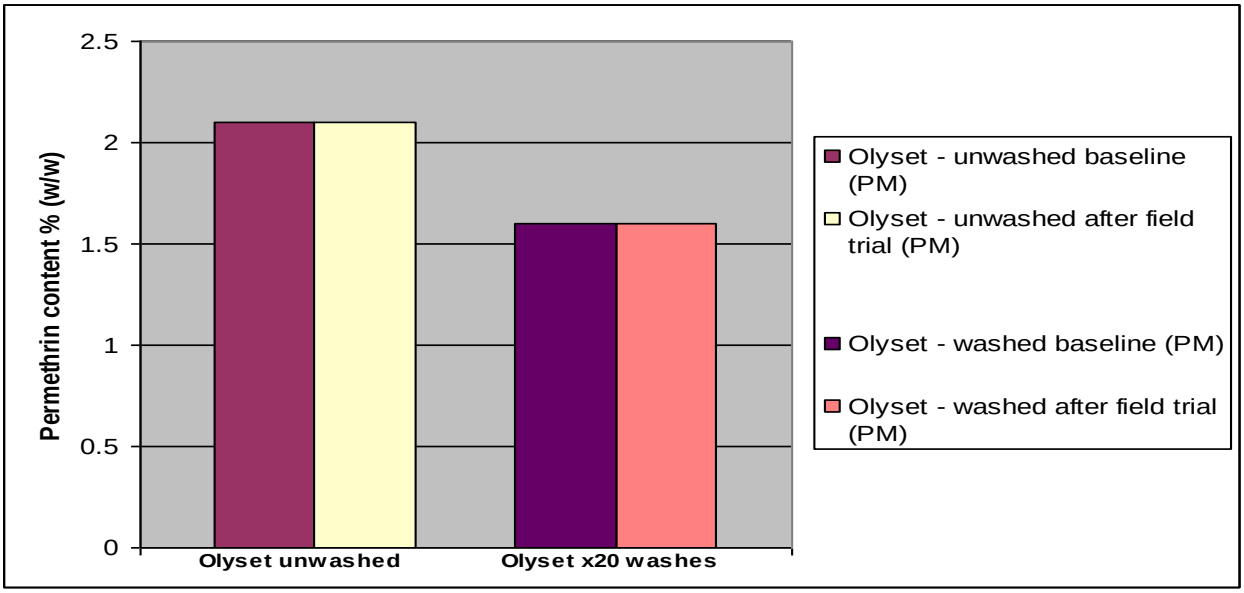


Fig 4B



Chemical analysis of net samples: Figure 4 shows the results of chemical analysis of residual deltamethrin content on the side panel of PermaNet® 3.0. The mean deltamethrin content of the unwashed side panel prior to and after field test was 85 mg/m^2 and fell to <43 mg/m^{2}after 20 washes but was still significantly higher than the CTN. The roof panel washed 20 times also lost 25% of the original (unwashed) baseline deltamethrin content and remained at this level post field trial. PBO content of the roof panel also declined from 25g/kg for unwashed net samples at baseline to 15g/kg after 20 washes and remained the same post field trial. The CTN samples had <65% of its original deltamethrin content after five washes. There was less decline in permethrin content of the Olyset® nets after 20 washes. The Olyset® nets still retained >75% of initial insecticide content and remained so at the end of the field trial (Figure 4).

Discussion

Proof of evidence of innovative resistance managementtools is needed from different

resistance spectrum prior to large scale field trials. In this study, both *kdr* and MFO-based resistance mechanisms were found in *Anopgeles gambiae s.s.* The synergist data and the increased level of monooxygenase in the resistant *Anopheles* population suggest the involvement of monooxygenase inpyrethroid metabolism. Laboratory cone bioassays using resistant *A. gambiae s.s.* prior to and after experimental hut evaluations showed high mortalityin PermaNet® 3.0, which was consistent with the field data.Increased bio-efficacy likely due to the synergistic effect of PBO and deltamethrin was obvious from *in situ* bioassays, with significant mortality of resistant *A. gambiae s.s.* recorded following exposure to the roof panel. This property however declined in bioassays with *C. quinquefasciatus* after 20 washes.

There is currently limited information on insecticide regeneration of Olyset® that has made comparison of PermaNet® 3.0 with the Olyset® net difficult to conduct. While self regeneration of Olyset® has been reported to take up to 2 weeks at room temperature[27], Olyset® net held at 30°C elsewhere did not regenerate after the same period of time[27].The incubation protocol adapted for the Olyset® in the present study allows for full insecticide regeneration between each round of net washing as evident in laboratory bioassay of Olyset® net samples against the reference susceptible stain of *A. gambiaes.s.*Therefore, the decline in bio-efficacy observed after 20 repeated washes of the Olyset® net samples was likely not due to insufficient insecticide within the fibres of the net but probably to problems associated with bioavailability of active ingredient on the surface of the net.

Analysis of the twelve weeks experimental hut data showed that PermaNet® 3.0 induced a level of deterrence against *A. gambiae and C. quinquefasciatus*similar to that of the Olyset® net. Both nets still deterred hut entry after 20 successive washes. The similarity in deterrency of both nets could explain the negligible difference in the overall number of mosquito collected in the different treatment arms. The excito-repellency property of both net types was also similar, but the feeding rate of *A. gambiaes.s*. in huts with PermaNet® 3.0 was highly minimized and the protective effect was not lost after 20 washes compared with the Olyset® net. The overall proportion of *A. gambiaes.s*. that successfully blood fed in the Olyset® net was triple that for PermaNet® 3.0. Significantly more *A. gambiae s.s.* was recorded blood fed with the CTNs washed to just before exhaustion. This result suggests that vectorcontrol with the Olyset® net or CTNs in the study site will be undermined by pyrethroid resistance as previously reported in neighbouring Benin Republic with reduced efficacy of ITNs and IRS due to high level of *kdr* and metabolic resistance[3] . Mortality of *A. gambiae* and *C. quinquefasciatus* in the untreated net was negligible compared to the insecticide treatment arms thereby making a correct deduction of the overall insecticide effect on mosquitoes more reliable. Despite the presence of both *kdr* and MFO resistance mechanisms, the proportion of *C. quinquefasciatus* and *A. gambiae s.s.* killed by PermaNet® 3.0 was high, even with the washed samples containing <50% of the baseline deltamethrin content, indicating the higher efficacy of PermaNet® 3.0 compared with the Olyset® net. Although there was a slight decline in mortality after twenty repeated washes, mortality data were also consistent with the laboratory bioassay. Previous studies in West Africa[23] comparing PermaNet® 3.0 and PermaNet® 2.0 have shown a higher mosquito mortality in PermaNet® 3.0; linked to the high deltamethrin content relative to PermaNet® 2.0. The higher efficacy of PermaNet® 3.0 relative to the Olyset® in this study could be due to the higher rate of resistance against permethrin in this area. The fact that PermaNet® 3.0 outperformed the CTN by far, showed that PermaNet® 3.0 fulfilled the WHOPES criteria for an LLIN. It also suggests that despite the high level of deltamethrin resistance, PermaNet® 3.0 is performing better, which could be attributed to the higher deltamethrin content as previously noted[23] or the presence of PBO in the roof, or a combination of these two features.

Taken together, our data revealed that PermaNet® 3.0 showed an increase efficacy over the CTNs and fulfilled the WHOPES criteria for LLINs and outperformed the Olyset® against resistant *A. gambiae s.s* and *C. quinquefasciatus.* With the increasing spread of pyrethroid resistance in African malaria mosquitoes, care must be taken to evaluate and monitor tools to find out if they have an effect on resistance. In the absence of such a tool, it is unlikely that LLINs will continue to provide the much needed protection in areas with insecticide resistance.

Conclusion

This study demonstrated under experimental field conditions that PermaNet® 3.0outperformed theOlyset® net and could provide additional protection in terms of reduction in blood feeding and increase in mosquito mortality: a plausible tool against pyrethroid resistant mosquitoes.

Ethics and conflict of interest: The study was approved by the Institutional Review Board and

the Research Ethics Committee of the Nigerian Institute of Medical Research and funded by Vestergaard Frandsen, Switzerland. The authors followed the WHOPES guideline for phase II evaluation of LLINs and have no commercial interest with the net manufacturers.

Acknowledgements
The authors wish to thank Mr. M.O. Ogungbemi and Charles Duker at the NIMR outstation for field assistance and the volunteers who participated as sleepers in the huts. We are grateful to Tessa Knox and Helen Pates Jamet for advice during the planning phase of the experiment.

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Nigerian Journal of Clinical & Biomedical Research (Jun. 2012) Vol. 6 No.1 44 – 46

OriginalArticle

Prevention of Mother to Child transmission services at the Lagos Island Maternity Hospital: The Journey so far

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Summary

Mother to child transmission of Human Immunodeficiency Virus (HIV) is the passage of HIV from an HIV positive mother to an infant.This can occur during pregnancy, labour and delivery and also during breastfeeding.This study was done to evaluate and review the outcome of Prevention of mother to child transmission of HIV (PMTCT), in Lagos Island Maternity Hospital for a period of five years (2005 – 2010). Data for this were extracted from the ante-natal clinic (ANC) register analyzed. Of a total of 10,924 pregnant women who registered for antenatal and care and had HIV counseling and testing during the study period;869(7.95%) tested positive for HIV. Results were not available for 1,527(13.97%) women. A total of 472(54.31%) of 869 HIV positive mothers were followed up. Out of those followed, 440 babies tested negative for HIV (93.22%), while 18 babies tested positive for HIV (3.81%). This study has demonstrated that the sero-prevalence rate of HIV in Lagos Island Maternity Hospital is higher than the National average. Intervention has revealed significant reduction in risk of transmission of the virus from mother to child. The attrition rate of positive mothers is quite high hence a more efficient means of follow up should be instituted.

Key words: Prevention of Mother to child Transmission, HIV/AIDS

Introduction

The risk of mother to child transmission of HIV is an unfortunate reality in many parts of Sub-Saharan Africa as access to measures aimed at prevention of mother to child transmission (PMTCT) is very limited.

Nearly 70% of those living with HIV in middle and low income countries do not have access to scientifically proven interventions to prevent mother to child transmission of the virus[1,2]. The major impact of PMTCT is felt through improving access to PMTCT care and expanding coverage[3].WHO has emphasized a sub-national maternal health services that focus on counselling pregnant women about HIV infection, and offering them HIV testing with a plan to provide prevention services to those who turn out with positive results[4]. These services usually involve provision of anti-retroviral drug, breastfeeding counselling and supplemental feeding and care for the new-borns of HIV positive pregnant women, while also integrating them into other reproductive health services. The pattern of transmission of HIV from mother to child if no intervention is done is such that 5-10% gets infected during pregnancy, about 10% get infected during labour and delivery, while about 5-15% get infected during breastfeeding and 60-75% will be born negative[5].

Lagos Island Maternity hospital commenced PMTCT services in 2005 through funding support from PEPFAR funded Global HIV/AIDS Initiative Nigeria. This study was done to evaluate and review the outcome of PMTCT services in the facility.

Methods

A 66 months (July 2005 – December 2010) retrospective study of PMTCT services at Lagos Island Maternity Hospital since the commencement of the PMTCT programme at the

center. During the period of review Voluntary counseling and Testing (VCT) in LIMH is performed using the "Opt Out" method, and as such, all the patients that attend antenatal care in LIMH are counseled and tested. PMTCT service information were extracted from the antenatal registers and case notes of the women. Extracted information were analysed with Excel software enhance with megastat.

Results

A total of 10924 antenatal attendes had VCT during the period. Of which a total of 869(7.95%) antenatal attendees tested positive for HIV, while a total of 8,528(78.0%) tested negative for the virus. 1,527(14.0%) did not have results. Four thousand five hundred and seventy two (54.3%) HIV positive mothers were followed up, while 397(45.7%) were lost to follow up. Out of 4572 women that were followed up, 440 babies tested negative for HIV (93.2%), while 18 babies tested positive for HIV (3.8%). Result of the remaining 14(2.96%) could not be traced.

Table 1 below shows the trend in number of pregnant women screened for HIV and the outcome of the results. The number of women that attended antenatal care services and screened for HIV steadily increased up to 2010 when there was a decline. While HIV positivity rate decreased from 8.5% in 2005 to 6.7% in 2009, the number of women with unknown result increased from 238(11.8%) in 2005 to 588(22.7) women in 2009. There was a dramatic decrease in the number of women with unknown result in 2010 (4.5%), with an increase in HIV positive rate of 8.0%.

The infant outcome of PMTCT services is shown in table 2. Of the 869 positive pregnant women seen during the period and had VCT, 397(45.7%) were lost to follow up and the infant HIV status was not known. Fourteen (1.4%) of the 472 women that were follow up had no results. Eighteen (3.9%) of the infant with test results tested positive to HIV. The Infant HIV positive rate decreased from 6.0% in 2005 to 3.2% in 2010.

Table1: Trend in antenatal care attendance and HIV test results in LIMH (2005 -2010)

Year	ANC Attendance	Negative (%)	Positive (%)	Unknown (%)
2006	2013	1604(79.7)	171(8.5)	238(11.8)
2007	2246	1759(78.3)	199(8.7)	288(12.8)
2008	2375	1841(77.5)	190(7.9)	344(14.5)
2009	2586	1833(70.8)	173(6.7)	588(22.7)
2010	1704	1491(87.5)	136(8.0)	77(4.5)
Total	**10924**	**8528(78.1)**	**869(7.9)**	**1527(14.0)**

Table 2: Trends in the infant HIV outcome during the study period (2005 – 2009)

Year	Positive mothers (%)	Negative babies (%)	Positive babies (% among infants with result)	No results(%)	Lost to follow up (%)
2007	199	126 (63.3)	8(6.0)	1	64(32.2)
2008	190	133(70.0)	2(1.5)	1	54(28.4)
2009	173	91(52.6)	5(5.2)	9	68(39.3)
2010	136	90(66.2)	3(3.2)	3	41(30.1)
TOTAL	869	440(50.6%)	18(3.9)	14(1.6)	397(45.7)

Discussion

The period under review from 2005 to 2010, revealed an HIV prevalence rate of 7.9% among the mothers tested. This is lower than that reported by Sagay and co-workers in Jos, Nigeria, but higher than the National average of 4.1.[6] A total of 1,527 of the 10,924 pregnant women seen and tested for HIV during the period had no results and as such their HIV status could not be determined. This represented 14.0% of the population studied. This is disturbingly high. This relatively high percentage of unretrieved result, will lead to inadequate data retrieval and interpretation. This in turn leads to poor interpretation of service coverage for PMTCT[7].The possible explanation for this poor retrieval of result could be the disconnect between the Care givers in the Antenatal clinic and the team who offers Voluntary counseling and Testing, as the Pregnant women receive antenatal care in a place different from where they have VCT done.

The follow up for HIV positive mothers was also poor in the study as only 54.31% of HIV positive mothers had follow up visits(n=472), thus by implication as much as 46.7% of the HIV positive mothers were lost to follow up. These tend to negate the fourth prong in the four prong approach of the strategic elements to PMTCT.

In the follow up of the HIV positive mothers, out of a total of 472 mothers followed up, 430 mothers had babies who were HIV negative, constituting 93.2%.A total of 18 mothers had babies who were negative for HIV, giving a a MTCT rate of percentage of 3.9%. As much as 2.9% did not produce results for their babies(n=14).The HIV positive rate of 3.9% is similar to that obtained in a work in South Africa[8].The use of dual therapy and HAART has been associated with lower mother to child transmission[9].A higher mother to child transmission of 66.6% was documented in a series published in India were single dose Nevirapine was used about 2hours before surgery.[10] In the period under review, the use of HAART and some cases dual therapy in this centre may have accounted for the relatively low level of transmission as compared to the India study. Lower rates of transmission have been documented in some other studies however[9].

Conclusion

The PMTCT programme in the Lagos Island Maternity Hospital has been proven to be effective with HIV transmission rate to Infants of Positive Mothers estimated to be 3.9%.The attrition rate is high across the programme.A more efficient means of tracking patients should be adopted and this should include retrieval of cell phone numbers, contact address and the phone numbers of close relatives.

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Nigerian Journal of Clinical & Biomedical Research (Jun. 2012) Vol. 6 No.1 47 – 53

Original Articles

Vaginal products and hygiene practices: implications for Microbicides acceptability amongst Nigerian women

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Summary

Use of vaginal products and or vaginal practices in cleansing, tightening and or drying the vagina could interfere with the use of newly initiated HIV preventive technologies for women.This study assessed vaginal practices, the use of other vaginal products and its implications on the acceptability of microbicides among Nigerian women. Three hundred and seventy women aged 19-45 were randomly selected and interviewed about vaginal practices, using questionnaire.Each participant completed a questionnaire to provide information on demography, knowledge and use of male/female condom, vaginal hygiene practices and use of intra-vaginal products. Also, Knowledge and willingness to use microbicides when available were assessed. This information was collated and analysedusing EPI INFO 2002 software.Of the 370 respondents, 51.6% were married**,** 61.4% had tertiary education. At baseline, vaginal douching was practiced by 77.8% of the women. The commonest agent used for douching was soap and water. Two out of 40% of HIV positive women reported having bruises after douching with lemon juice. A total of 12.4% of the women reported inserting tightening substances to increase sexual pleasure. Use of tightening substances and douching practices were not significantly associated with microbicide acceptability ($p > 0.335$, $p > 0.609$). Contrarily, 49.7% women who use lubricated inserts was significantly associated with microbicide acceptability $p<0.05$. Willingness to accept and use micobicides were also significantly associated with previous knowledge, education, religion, age, marital status as well as family planning methods($p<005$). The use of lubricated inserts may indicate willingness to accept and use hypothetical microbicides, contrarily, use of tightening substances may indicate negative implication for microbicides and calls for public health intervention.

Key words: Vagina, hygiene**, v**aginal products**,** microbicides**, w**omen

Introduction

Vaginal douching or washing is the process of vaginal cleansing with a liquid solution. It is practiced by millions of women around the world[1] (. In Africa, the prevalence ranges from 6 to 98%[2] . Naturally, women's genital hygiene is highly valued but there are issues regarding cleanliness, and related vaginal practices which has been generating conflicting views on the benefits or side effects of douching. Most women who douche consider it to be a healthy practice. The major reasons why women douche are to maintain their vaginal hygiene which includes: cleaning up after menstruations, sex, treat vaginalsymptoms such as itching and discharge, to prevent pregnancy or sexually transmitted diseases as well as to improve health-self treatment for odour. Also, to meet expectations or preferences of sexual partners as well as follow traditional norms.

Variety of liquid solutions and solid substances are used as they differ from one geographical region to

another. These include dettol (disinfectant), antiseptic soap, mixture of local herbs, water alone, alum, snuff, finger, lemon juice, and other chemical douching products which are commonly used in Nigeria, Zimbabwe, Cameroon and South Africa[3,4,5].

Again, it is interesting to know that in African countries, virginal products especiallynatural herbs, alum, leaves, Vaseline, cloth, paper or cotton wool are used by women to ensure warmness, tightness, closeness, or dryness of the vagina in order to improve sexual relationships[6] (.These women prefer dry sex irrespective of their social status. However, others see wetness or lubrication as an indication of looseness, infidelity or diseases[7,8]. Studies have shown that vaginal practices have been linked to loss of lactobacilli, cause disruption of membranes lining the vagina and uterine wall as well as increase the pH of the vagina encouraging growth of bacterial vaginosis, a condition shown to increase women's risk of HIV infection and pelvic inflammatory diseases[2,9]. Kubukeli in 1998, reported that dry sex practices is the most prevalent in Kwazulu-Natal which has the highest incidence of HIV/AIDS[10]. Excessive drying could also lead to abrasive trauma during sexual intercourse. These dry techniques compromise the effective use of male and female condoms, and may promote the spread of HIV and other STIs. Due to global burden of HIV and its increasing feminization and the high practice of vaginal cleansing, tightening or drying, it becomes necessary to understand whether these practices will interfere with acceptability and use of the currently being evaluated preventive technologies such as microbicides, diaphragms, cervical barriers films, vaginal rings, fast-dissolving vaginal tablets and novel polymers being investigated[11]. Again, local or chemically produced vaginal products require insertion into the vagina in preparation for sex which may alter the activity of microbicide as noted by Hilber *et al*[12](2007) who stated that social norms about lubrication during sex and parallel vaginal practices have significant implications for acceptability of microbicides that modify vaginal lubrication.Smith's study[3] in a Microbicides trial site in South Africa observed that 'Cultural rituals pose challenges to microbicides clinical trials as women combine the gel with other barriers to HIV including dettol (antiseptic), herbs, snuff and lemon juice'.

This study therefore aimed at identifying the type of vaginal practices that are common amongst women of reproductive age and also determine whether the use of vaginal inserts before sex can influence vaginal microbicide acceptability among Nigerian women.

Methods

Study design: The study was conducted from 2006-2008 using a cross-sectional study design. Random sampling of HIV seropositive women (HIVPW) from HIV treatment centre, Nigerian Institute of Medical Research, Market women (MW), Female workers in Nigerian Institute of Medical Research (FWNIMR), Church women from some churches in Lagos (CHW), as well as students from University of Lagos (SUNILAG) were interviewed. A detailed questionnaire was administered privately by same sex mostly in English and local pidgin language. Three hundred and seventy women who gave oral consent were interviewed. Their age range was between 19 and 45 years, as well as sexually active and of reproductive age. They were made to understand the benefits and risks involved in the study.

Data collection: Three hundred and seventy women who were sexually active and of reproductive age were randomly selected and interviewed using detailed semi-structured questionnaire. Each participant completed a questionnaire in order to provide demographical data. Information on the use of male (bypartner) and female condoms, how often the women get cooperation from their partners to use male condom or female condom and the use of any other lubricant was obtained. Their knowledge and willingness to accept and use microbicides when available was ascertained. Information on types of products used to warm, dry and tighten the vagina was obtained. Information on types of substances used to prevent pregnancies and vaginal infection before and after menstrual period was obtained. Other questions covered hygiene practices which include: Absorbent materials used for menstrual period, bathing frequency during menstrual period, cleanup process after sex, douching products used and frequency of douching.

Data analysis: The data obtained were entered and analysed using EPI INFO 2002 software (CDC, USA). Chi square statistical analysis was used to test the significance between vaginal hygiene practices (douching or washing), use of vaginal products (tightening, drying and warming), and the implications on microbicides acceptability and use. Correlation between age, religion, education, marital status, condom use (male and female), vaginal products and willingness to accept and use microbicides were determined by chi square where appropriate. Level of statistical significance for all analyses was set at $p< 0.05$.

Results

Study population: Of the 370 respondents studied, One hundred and ninety one 191 (51.6%) were married, 146 (39.5%) single, 27 (7.3%) divorced and 6 (1.6%) widowed. In addition, 227 (61.4%) had tertiary education, 90 (24.3%) had secondary education, 5 (1.4) had primary education while 4 (1.1%) had no education at all. Majority of the respondents 286 (77.3%), were Christians, 75 (30.3%) were Moslems, traditional religionists were 8 (2.2%), and others 1 (0.3%). The professional backgrounds of the respondents included: Health Workers 99 (26.8%), Artisans 83 (12.4%), House wives 28 (7.6%), Bankers 24 (6.5%) and Teachers and Traders 68 (18.4%) respectively.

Knowledge and use of male, female condoms and other lubricants, and willingness to accept and use Microbicides: In order to determine the Knowledge/use of male and female condoms*vs.*knowledge/willingness to accept and use Microbicides when available: it was observed that340 (91.9%) of the respondents have knowledge of male condom while 30 (8.1) do not. 187 (50.5%) accepted that their partners use condom while 183 (49.5%) did not. Two hundred and nineteen (59.2%) insist that their partners should use condom, while 169 (45.7%) receives co-operation to use condom from their partners. Meanwhile, 78 (21.1%), use condom consistently. Of the 370 respondents, 71 (19.2%) accepted that their partners use other lubricants other than condom. Meanwhile, Two hundred and sixty (70.3%) have knowledge of the female condom while only 35 (9.5%) use it. Two hundred and thirty (62.2%) have previous knowledge of microbicides while 235 (63.5%) of the respondents would like to use the product, when it is available in the market. However, previous knowledge and use of male, female condoms and other lubricants were significantly associated with willingness to accept and use microbicides. Chisquared P= 0.002(table 1).

Douching frequencies and substances used for douching: Though a total of 288(77.8%) accepted to have practiced douching, a slightly smaller number - 240(64.9%) douche on a daily basis. The remaining 48 of the 288 that douched do that either on weekly (26) or monthly (22) basis. (soap and water 120 (32.4%), antiseptic (Dettol) 96 (25.9%), lime and lemon 73 (19.7%), finger and water 56(15.7%) and alum and water 23(6.2%) were the substances used for douching.Majority reported leaning the practices from their mothers (42.2%) friends (13.8%) and relatives (4.1%). Further analysis showed that douching practices were not significantly associated with willingness to use microbicide (P=0.61).

Use of vaginal tightening substances, herbs and association with acceptance of microbicide: Table 2 shows the use of vaginal tightening substance among the respondents. While 46 (12.4%) of the respondent reported having used vaginal tightening substance before sex, 27(7.3%) respondents inserts herbs into the vaginal after sex for cleansing purposes. Alum (95.7%) was the most common substance used for vaginal tightening among the respondents who used tightening agents. The substances used for vaginal tightening were Further analysis showed that use of vaginal tightening agents and herbs were not associated with willingness to use microbicide (p = 0.335).

Substances used to terminate pregnancies and other family planning: The respondent employed various means of prevention or termination of unwanted pregnancies. Of the 370 women respondent, 8(2.2%) use alum, 10(2.7%) use Schweppes (a commercial lemon mineral drink), 38(10.3%) use concentrated lemon and lime fruit. Thirty six (9.7%) use herbs made of *Piper Guinness* (Uziza seed or leaves), *Myristica fragrance* (Ehuru fruit) and *Xylopia aethipica* (Uda fruit) to prevent or abort their pregnancies. The four local herbs are usually mashed and boiled, and the broth mixed with alcohol. Seventy two (19.5%) respondents use condom, while 38(10.3%) use Andrews liver salt (also made of lime and lemon) mixed in warm water. One hundred and twenty six (34%) use family planning methods. Most of the women, who do not want to use any other family planning methods, get their partners' to use natural methods such as ejaculating outside the body (16.2%). Twenty (5.4%) are either not married or are virgins. There was a significant association between the use of these substances and acceptance and use of microbicides. Chisquared p=0.0031.

Other hygiene practices: This study also identified other Hygiene practices the respondents were involved in. Of 370 respondents, 339 (91.7%) bath twice daily while 31 (8.3%) bath once daily during menstrual period. Two hundred and seventy five (74.3%) use commercial lady's pad as absorbent material during menstrual period, 51 (13.8%) use tissue paper, 29 (7.9%) use cotton wool or cloth while 15 (4.0%) use tampon. Among those who use tissue, some reported having heavy flow but they make several changes of tissue. The point is that

Table 1. Knowledge and use of Male and Female Condom and other lubricants

Question on Knowledge and use	No of Respondents –n=370 (%)
Heard of male condom	
• **Yes**	340(91.9)
• **No**	30(8.1)
Does your partner use it	
• **Yes**	187(50.5)
• **No**	183(49.5)
Do you insist on usage?	
• **Yes**	219(59.2)
• **No**	151(40.8)
• **N/A**	8(3.5)
If you do does he cooperate?	
• **Yes**	169(45.7)
• **No**	201(54.3)
How often does he use male condom?	
• **Always**	78(21.1)
• **Sometimes**	123(33.2)
• **Don't use**	168(45.7)
Use of gel, vaseline, foam. Spong	
• **Yes**	71(19.2)
• **No**	299(80.8)
Heard of female condom?	
• **Yes**	260(70.3)
• **No**	110(29.7)
Do you use it?	
• **Yes**	35(9.5)
• **No**	335(90.5)
Heard of Microbicides?	
• **Yes**	230(62.2)
• **No**	140(37.8)
Willing to use it?	
• **Yes**	235(63.5)
• **No**	135(36.5)

Table 4: The use of vaginal tightening substances and herbs among the respondents.

Substances /Herbs	No of respondents (%)	Time of Insertion
Vaginal tightening	46(12.4%)	
• Alum	44(95.7)	Before sex
• Miss Flora tightening	1(2.2)	Before sex
• Super grip (Love forever)	1(2.2)	Before sex
Herb	27(7.3)	After sex
Never tightening agent /herb	297(80.3)	

they are not inconvenienced as long as the tissue is flushed in the toilet. However, 183(49.5%) of the respondents use soap and water to clean up after sexual act, 118(31.9%) use water only while 69(18.6%) use only tissue (table 3).Respondents who used various substances to prevent infections after sexual activity and menstrual periodwere determined as follows: clean up with antiseptic soap, warm water and salt, 46(12.5%), herb and alum 23 (6.2 %) respectively. Furthermore, 75.1% of the respondents reported using antibiotics when they observed itching and abnormal vaginal discharge after menstrual period. This resulted to their application of the following vaginal insertion creams: Trosyd (4.9%), cannesten (4.1%) and nystatin (4.1%).

Table 3: Absorbent materials used for menstruation, cleaning materials used during menstruation and sexual activity

Menstrual Practices	No of Respondents (%)
Absorbent materials	
• **Pad**	• 275(74.3)
• **Cotton wool**	• 18(4.8)
• **Cloth**	• 11(2.9)
• **Tampon**	• 15(4.0)
• **Tissue**	• 51(13.7)
No. of bathing times	
• **Once**	• 22(6.0)
• **Twice**	• 339(91.6)
• **Did not indicate**	• 9(2.4)
Clean up after sex	
• **Soap and water**	• 183(49.5)
• **Water only**	• 118(31.9)
• **Tissue**	• 69(18.6)

Association between select variables and willingness to use microbicide: Of variables assessed for any correlation with willingness to use microbicide, only age group 21-35 years(p=0.001), being educated up to tertiary level(P=0.001) and Christian religion(P=0.03) were found to be significant associated with willingness to use microbicide .

Discussion

The use of vaginal products and vaginal hygiene practices and its implications in accepting microbicides among Nigerian women is of absolute importance. The study gives an overview of vaginal practices and its implication in the acceptability and use of vaginal microbicides in Nigeria.

From our study a significant number 49.7% of women are already using lubricated vaginal inserts before sexual activity. Since there has been no notable adverse reaction in the use of these lubricants, (male and female condoms, gel, Vaseline, diaphram) it will be expected that by introducing safer and affordable microbicides which could offer protection against HIV and other STIs, would be preferred to the lubricants the respondents are using (P<0.05) .

In this study, however, the acceptability of microbicides may not likely be applicable to 12.4%women who engage in tightening or drying their vagina before sex with substances such as alum, herb, love and others (p>0.05).Vaginal drying agents are used when there is a cultural preference for dryness in place of a lubricated vagina. Tight and dry vagina could cause disruption of membranes lining the vagina and the uterine wall, causing abrasive trauma during sexual intercourse. This potential harmful practice is most likely to be associated with increased risk of HIV acquisition.From our study,64.9% of Nigerian women douche once daily. In another recent report by Otuonye *et al*[5], (2011), douching was significantly associated with acquisition of HIV and other STIs in a cohort of 450 Female Sex Workers (FSW) in Lagos Nigeria.The FSW who douched had 21% chance (risk) of acquiring HIV/STIs than those who do not douche.Another study done by Christine *et al*[13] (2008) showed that intravaginal use of 100% lime juice for douching is associated with large colposcopic findings that have not been conclusively linked to HIV acquisition. Other previous studies reported that douching has been associated with many adverse outcomes including pelvic inflammatory diseasesand ectopic pregnancy. Judging from the National HIV prevalence, HIV infection in Nigerian women is 4.0% compared to their male counterparts 3.2%[14] (National HIV/AIDS and Reproductive Health Survey, 2007). It has therefore become necessary to target women who are involved in douching practices in sexual health awareness programmes. Information on reducing douching practices may go a long way to reduce virginal infections and may reduce the rate of HIV and other STIs acquisition in sexually active and reproductive aged women in Nigeria.

It was also observed that30% of the respondents in this study had experienced pain and or discomforts but have never linked it to the substances they used regularly for douching or

inserted before or after sex.Interestingly, few of them (10%) stopped douching after the interviews we had with them. Some who reported back acknowledged that they had a lot of relief from their pain though; they admitted achieving the purpose of preventing vaginal infections and or pregnancy. Further studies are needed on the effect of these substances on Human immunodefficency virus and at what concentration disinfection can be achieved *invivo* to prevent HIV infection with no harmful effect to the vaginal epithelium. This finding is consistent with the study done by Imade *et al*[15], who worked on the use of lime/lemon juice for douching by women in Jos, Nigeria. In that study 19% of the women were reported to have pains but could not be linked to douching with lemon/lime juice or pre-existing vaginal lesions. However, in this study, douching was not significantly associated with microbicide acceptability and use p=0.609.

Respondents in this study used local substance as contraceptives such as Uziza (*Piper guinenses* also called womb cleanser), Uda fruit (*Xylopia aethipica*: an analgesic/stimulant) and others in alcohol. How the chemical composition of these substances prevents or terminates pregnancies is yet to be understood and may require further studies. It is likely that the 24% of the women who engaged in native (harmful) practices in protecting themselves against pregnancy may be more willing to accept contraceptive microbicides p<0.05.

The age group, 21-35 in this study showed that this group of reproductive aged women are more sexually active and more at risk of HIV/STIs infections. The group are also significantly associated with willingness to accept and use microbicides (p=0.0013). The factors related to this increase in this group include more sexual activity, low condom use, increased number of sexual partners, socio-economic factors and personal hygiene. This result is comparable to previous studies done by Otuonye *et al*[16], (2004). They observed that STI/HIV were more prevalent between ages 20-30 years than any other age group.

Christians (73%) were more willing to accept microbicides unlike other religious group *(p=0.03),* however, Nigerian tribes were not associated with microbicide acceptability and use.

Again, the respondents who have tertiary education were more willing to accept and use microbicides, (56.2%, p=0.0065). Also, prior knowledge of microbicides (81%, p=0.0001) increased the willingness to accept vaginal microbicides. Understandably, single and married women are more willing to accept and use microbicides. These groups of young women are more sexually active, more at risk of HIV/STIs, as well as, have increased number of sexual partners. It is commendable to note that 80% of the study population practice good hygiene (bathing twice daily) during menstrual PERIOD and proper cleaning up after sex irrespective of their level of education and profession. The good hygiene practices seem to have cut across almost all ethnic groups in Nigeria represented in the study.

Conclusion

This study has shown the likelihood of acceptability of microbicide as a significant, percentages already practice the use of lubricated inserts. The women (12.4%) who engage in drying and tightening prior to sexual activity may have need to be enlightened about the disadvantage of harmful substances. They are already compromising condom integrity by rendering it inadequate as protection for STIs, HIV and contraception. This may have a negative implication for microbicides and also calls for urgent public health intervention.

There is need to investigate these natural substances to determine whether its use should be promoted or discouraged since some of them have been confirmed to disrupt vaginal integrity leading to other vaginal infections and acquisition of HIV due to abrasion, irritation and or inflammation. It is also worthy to note that 40% of the respondents were HIV positive. We could not determine whether the infection was acquired as a result of their douching habit or the vaginal tightening products they have been using or through other means.

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Nigerian Journal of Clinical & Biomedical Research (Jun. 2012) Vol. 6 No.1 54 – 59

Original Article

In-Vitro Inhibitory Activity of Lactodouche on the Growth of Candida SPP and Other Uropathogens

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Summary

In search of ecofriendly, affordable and enduring solution to persistent candida vaginitis, Lactodouche, a douching agent was formulated and prepared from *Lactobacillus* species. *Lactobacillus* species were isolated from vaginal swabs and African fermented foods as starter cultures. *Candida albicans* were cultured from vaginal swabs only. Assay of bacteriocin and other antibacterial agents were done by the agar spot method. Inhibitory activities were carried out on *C. albicans* and other uropathogens. Identification and characterization were done using standard laboratory methods. Of the total 935 females, 255 (27.3%) had symptomatic candidal vaginitis while 680 (72.7%) were asymptomatic. Of these, *L. acidophilus* 187 (27.5%) was the most common isolate from the two groups of women, occurring in 72(10.6%) of the women as a single species. The agar spot method and liquid suspension of antibacterial agents produced by *Lactobacillus* both recorded inhibitory activities on the growth of *C. albicans, S. aureus* and *E. coli* .The *Lactodouche* formulated using each strain of NM30 and NM 72 had inhibitory activity on *C. albicans.* Each filter sterilized aliquots of *L. acidophilus* and *L. plantarum* expressed zones of 20mm, and 30mm diameters respectively against *C. albicans*. The study shows that lactobacillus and lactodouche has inhibitory activity on the growth of *C. albicans, S. aureus* and *E. coli* invitro.

Keywords: Bacteriocin, Candidiasis, Inhibitory, Lactobacillus, Lactodouche

Introduction

Since Metchnikoff[1] proposed a role for *Lactobacillus* in suppressing undesirable intestinal microflora, numerous researchers have investigated the antimicrobial activities of *Lactobacillus species.* Broad-spectrum inhibition has been clearly demonstrated for bacteriocins, organic acids and hydrogen peroxide produced by *Lactobacillus spp*[2]. These substances have been shown to have inhibitory activities against numerous bacterial genera including *Proteus, Salmonella, Streptococcus and Bacillus*[3] . Such antibacterial activities have demonstrated the usefulness of *Lactobacillus* in human health.

Although the usual microbiota of the vagina from menarche to menopause is dominated by *Lactobacillus*[4], many other organisms can be cultivated from vaginal samples[5,6] . Among such organisms is *Candida albicans* known to cause vaginal thrush with excoriating, pruritus and erythematous syndrome.

Candidal infection is a common infection and major cause of ill health and discomfort. Attempt at controlling the infection have failed not only as a result of non-compliance to antifungal drugs and messy nature of available topical antifungal, but the emergence of resistance to the commonly available antifungal agents[7,8,9,10].

Emerging evidence from Nigeria and elsewhere had reported the antibacterial properties of substances produced by *Lactobacillus*[7,8]. There is

therefore the possibility that it may control other common vaginal infections like candidiasis.
This study was primarily designed to evaluate the in-vitro activity of Lactobacillus against *Candida spp.* and other uropathogens.

Materials and Method

Study Population: A total of 935 females attending antenatal, post natal, fertility and outpatient clinics of the Lagos University Teaching Hospital (LUTH) and the Nigerian Institute of Medical Research, Lagos who gave their consent were enrolled into the study. A total of 1235 vaginal swab samples were collected before and after administering vaginal pessaries. Laboratory investigations were carried out in the Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, Yaba, Lagos

Culture, Identification and characterization: For the isolation of *Lactobacillus*[11], vaginal swab samples were cultured on De Man Rogosa and Sharpe Agar (MRSA) (CM361 – Unipath Ltd, Hampshire). Isolation of yeast was done on Sabouraud Dextrose Agar (SDA: CM362 – Oxoid). Plates were incubated under microaerophilic conditions making use of candle extinction jar at 37°C for 18-24 hrs.
Standard laboratory method as enunciated by Cowan and Steel [12]were used in the identification of isolates of *Lactobacillus, Candida albicans* and other bacterial pathogens isolated.

Assay of bacteriocin and other antibacterial agents: Bacteriocins and other antibacterial agents were assayed by the agar spot method [13]. This was carried out consecutively for four days. **Day1:** Fresh growing *Lactobacillus* isolate was inoculated into MRS broth containing 5% glucose and incubated in a candle jar (10% CO_2) at 37°C for 18-24 hrs. **Day 2:** 5 µL of inoculum of *Lactobacillus* (producer strain) was inoculated unto the center of dried MRSA plate and incubated overnight at 37°C. Nutrient broth (CMI 341 – Oxoid) was also inoculated with *Candida albicans* (indicator organism) and incubated overnight at 37°C. **Day 3:** 100-150 µL inoculum of *C. albicans* was added to 5 mL semi-solid nutrient agar and carefully poured over spotted producer strain and incubated aerobically overnight. **Day 4:** Zones of inhibition were measured with a ruler.

Characterization of inhibitory substances: Inhibition caused by hydrogen peroxide, lactic aid and bacteriocin were identified. Bacteriocin producing *Lactobacilli* were identified by formation of a clear halo on the medium as described by Olukoya and colleagues [14]. The inhibitory activity caused by organic acids were excluded by buffering while hydrogen peroxide was removed by addition of catalase.

Growth of *Lactobacillus* in liquid medium: *Lactobacillus acidophilus* (k93), *L. salivarus* (k5) were grown in MRS broth in Eppendorf tubes and incubated in CO2 extinction jar for 18-24 hrs. Cells were harvested by centrifugation in a whirl mix centrifuge at 6000 rpm for 30 mins. Supernatant was decanted and filter-sterilized (0.25 µm pore size). The sensitive yeast strain was spread on an agar plate and 50-100 µL of producer strain was spotted onto this lawn. Plates were incubated aerobically at 37°C and zones of inhibition read after 18-24 hrs [15].

Analysis of Growth from vaginal samples: The various isolates obtained from each sample were recorded. Microbial growths of vaginal samples from pregnant and non-pregnant women were recorded. Females with clinical symptoms whose cultures yielded both *Lactobacillus* and yeast were also recorded.

Preparation of experimental sample of *Lactodouche:* *Lactobacillus* strains isolated from African fermented foods served as starter cultures. The fermented foods were Tapioca (*Manihot esculenta*: crantz), Kenke (*Zea mays*), and Kunu (Millet and Sorghum). Strains of *L. acidophilus* (NM30), *L. plantarium* (NM72) and *L. casei* (NM45) were isolated and used in the preparation of *Lactodouche.* These strains were shown to produce hydrogen peroxide, lactic acid and bacteriocins with activity against *C. albicans* and other uropathogens. Approximately 1 g wet weight producer strain NM30, NM45 and NM72 were each added to 100 ml of MRS broth and emulsified. The inoculum was incubated overnight in CO_2 extinction jar at 37°C. Thereafter, the broth was centrifuged at 6200 rpm for 30 mins. The decanted supernatant was filter-sterilized to give a clear solution. All antibacterial substances produced by *Lactobacillus* served as the douching agent. Where sterilization was done in the autoclave, bacteriocins remain the only active agent being thermostable [14]. The *Lactodouche* was packed in 200 mL soft plastic container with a hard plastic applicator. All preparations were done under sterile conditions. Work is on-going on the starter culture for large scale production of *Lactodouche.*

Organic acids and Hydrogen Peroxide in *Lactodouche:* A major organic acid identified in *Lactodouche* is lactic acid, with a pH of 5.5. The total filterable acidity was deduced by titrating 20

mL of aliquot against 0.1N NaOH to pH 8.3. Detectable hydrogen peroxide was shown in *Lactodouche* using catalase.

Data management: Information obtained were entered and analysis using EPIINFO 6.0. The p value was based on 95% Confidence Intervals ; a p value > 0.05 was not significant (NS).

Results

Relative Distribution of *Lactobacillus:* Of the total 935 females sampled 255 (27.3%) had symptomatic candidal vaginitis while 680 (72.7%) were asymptomatic. The asymptomatic females comprised of 360 pregnant and 320 non-pregnant women. The proportion of lactobacillus from pregnant and non-pregnant women are shown in Table 1. Of these, *L. acidophilus* 187 (27.5%) was the most common isolate from the two groups of women, occurring in 72(10.6%) of the women as a single species. This was followed by *L. casei,* 137(20.1%) and *L. salivarius,* 131 (19.3%) in these women. There was a significant association between *Lactobacillus spp* and pregnancy (P<0.01). *L. delbruckii, L. plantarum and L. jensenii* were isolated more in pregnant than in non-pregnant women; whereas *L. bifermentans and L. salivarius occurred more in non pregnant women.*

Co-existence of *Lactobacillus* with *Candida albicans* in asymptomatic individuals: In the asymptomatic group, there were no yeast cells without *Lactobacillus* being present. All the yeast cells were *Candida* spp, 79.2% were *Candida albicans* . Generally, the co-existence of *Lactobacillus* with yeast was low 120 (17.6%).*L. acidophilus* had the highest rate of co-existence with yeast, (26.7%) followed by *L. bifermentans* (18.2%).whereas *L. casei* had the lowest rate of co-existence (8.8%)(Table 2). Among the pregnant women, *L. casei* and *L. bifermentans* were not found coexisting with *Candida albicans.*

***Lactobacillus* and *Candida albicans* in Symptomatic patients:** Of the 255 symptomatic females, *L. acidophilus* , *L. plantarum* and *L. lactis were each 10(20-30)while L.casei* was the most single occurring *Lactobacillus* in pregnant, non-pregnant and randomized females respectively. An isolation rate of (20-60%) were recorded for *Candida albicans*. Rates for co-existence of *Candida albicans* and *Lactobacillus* species were generally low (20-40%). There was a significant growth of *Candida albicans* in symptomatic than in asymptomatic females (F=7.71; P<0.05).

Inhibitory activity of *Lactobacillus species against Candida albicans* and other uropathogens: The agar spot method and liquid suspension of antibacterial agents produced by *Lactobacillus* both recorded inhibitory activities on the growth of *C. albicans, S. aureus* and *E. coli* (Table 3).

Table 1: The proportion of *Lactobacillus* species isolated from pregnant and non-pregnant women

Lactobacillus spp.	Pregnant	Non-pregnant	Total
L. acidophilus	101 (54.0%)	86 (46.0%)	187 (27.5%)
L. bifermentans	34 (34.0%)	66 (66.0%)	100 (14.7%)
L. casei	75(54.7%)	62(45.3%)	137 (20.1%)
L. delbruckii	70 (60.9%)	45 (39.1%)	115 (16.9%)
L. jensenii	77 (59.2)	53 (40.8%)	130 (19.1%)
L. plantarum	77 (59.7%)	52 (40.3%)	129 (19.0%)
L. salivarius	52 (39.7%)	79 (60.3%)	131 (19.3%)

Chi square = 31.03 DF = 6 P<0.001

Table 2: Coexistence of *Lactobacillus* species with Yeast cells in asymptomatic group

Lactobacillus spp	*Lactobacillus and* Yeast	*Lactobacillus* alone	Total
L. acidophilus	50 (26.2%)	141 (73.8%)	191
L. bifermentans	16(18.2%)	72(81.8%)	88
L. casei	10(8.8%)	104(91.2%)	114
L. delbruckii	12(14.3%)	72(85.7%)	84
L. jensenii	10(11.2%)	79(88.8%)	89
L. plantarum	17(23.6%)	55(76.4%)	72
L. salivarius	5(11.9%)	37(88.1%)	42
Total	120(17.6%)	560(82.4%)	680

Table 3: Co-existence of *Candida albicans with lactobacillus* species in symptomatic patients

Category		Lactobacillus only (n = 60)	C. albicans only (n = 115)	Lactobacillus and C. albicans (n = 80)	Total
Pregnant	L. acidophilus	10(18.2%)	30(54.5%)	15 (27.3%)	55
Non-pregnant	L. plantarum	10(20.0%)	25 (50.0 %)	15 (30.0%)	50
Randomized	L. salivarus	10(20.0%)	20 (40.0%)	20 (40.0%)	50
Fertility Problem	L. casei	20(40.0%)	10 (20.0%)	20 (40.0%)	50
Undergraduates	L. lactis	10(20.0%)	30(60.0%)	10(20.0%)	50

The strains of *L. acidophilus* (NM30), *L. casei* (NM45) and *L. plantarum* (NM72) expressed inhibitory zones against *C. albicans* and other uropathogens. *L. acidophilus* expressed the widest zone of inhibition (20-35mm) against *C albicans. L. plantarum* expressedzones of 10-20mm against *C. albicans* both inagar and liquid suspension.

Table 4: Inhibition profile of *Lactobacillus* species on *Candida albicans* and other uropathogens

Lactobacilli	Agar spot method		Liquid suspension	
	C. albicans	*S. aureus*	*E. coli*	*C. albicans*
L. acidophilus (NM30)	+	++	+	+++
L. plantarum (NM72)	++	+	+	++
L. casei (NM45)	+	-	+	++
L. bifermentans (NM7)	+	++	-	+
L. lactis (K1)	+	++	+	++
L. salivarus (K5)	-	+	-	+
L. acidophilus (K93)	+	++	+	++

Zone diameter

+ 5-10 mm ++ 10-20 mm +++ 20-35 mm

NM strains isolated from fermented foods. K strains isolated from vagina

The narrow zones observed from agar spot method depicts presence of bacteriocin (Fig. 1) while the large zone in liquid suspension contained hydrogen peroxide and lactic acid (Fig. 2).

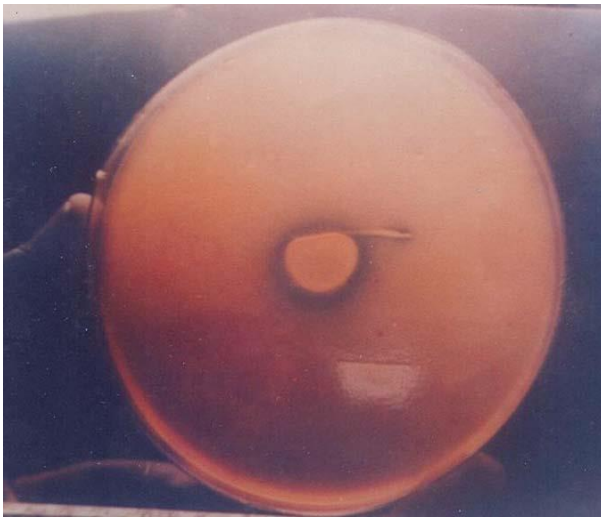

Fig 1: Inhibitory activity ofbacteriocin producing strain of *Lactobacillus acidophilus against .Candida albicans.*

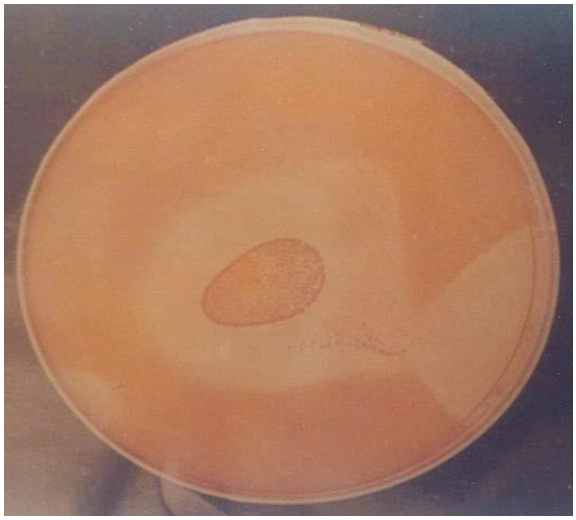

Fig 2: Inhibitory activity of liquid suspension of *Lactobacillus plantarum* against *Candida albicans*

Effect of Lactodouche on C albicans:
The *Lactodouche* formulated using each strain of NM30 and NM 72 had inhibitory activity on *C. albicans.* Each filter sterilized aliquots of *L. acidophilus* and *L. plantarum* expressed zones of 20mm, and 30mm diameters respectively against *C. albicans*. After heating the formula and testing against *C.albicans*, zones of inhibition measuring approximately 8mm in diameter were recorded indicating the presence of bacteriocins (Fig. 1).

Discussion
The health benefit of *Lactobacillus* in new born infants and other benefits related to blood cholesterol levels are generally accepted by medical community[15]. This usefulnesswas also shown when it was used in"Dogik" a weaning cereal with potentials for diarrhea control [18].Further more the health importance of lactobacillus was shown in "lactodouche" the proposed therapy of candidal vaginitis. Three *Lactobacillus* species (*L. acidophilus, L. plantarum and L. casei*) which inhibited *C. albicans, S. aureus* and *E. coli* were each useful for the production of the douching agent. *Lactodouche* was found to be effective when Seitz-filtered or heat sterilized since the bacteriocins present are thermostable. *Lactodouche* is safe in humans. The safety is attributed to the fact that *Lactobacillus* species, are normal flora of the human vagina.
The existence of *Lactobacillus* in the female vagina irrespective of the age group (17) was shown by the frequent isolation of a variety of *Lactobacillus* species including *L. acidophilus, L. plantarum, L. case, L. salivarus* and *L. lactis.* Besides, the frequent isolation from healthy females and few isolation from those diagnosed for Candidiasis is an indication of their protective properties.

In this study, the presence of *Candida albicans* as a single pathogen in females diagnosed for candidiasis simply showed the overwhelming effect of yeast on *Lactobacillus*. Colonization by yeast suppressed the protective effect given by *Lactobacillus*[18]. The low rates recorded for *Lactobacillus* in co-existence with *Candida* further compromised the protective properties of *Lactobacillus* in the vagina.

Another aspect are the zones of inhibition expressed by *Lactobacillus* against *C. albicans* and other uropathogens. This was shown by the agar spot method after establishing bacteriocins. Even where catalase was added to the medium, inhibitions were observed[19].

The causal agents of candidiasis and other bacterial pathogens present in the vagina are known to cause discomfort[20]. The symptoms include intense pruritus and erythema. Unfortunately, the use of pessaries has not solved the problem. Exploiting the inhibitory properties of *Lactobacillus* may give relief to candidal infection. Earlier studies have shown that inhibitory properties of *Lactobacillus* were useful in controlling diarrhoegenic agents[8] and other bacterial pathogens[7], thus confirming the results of this study.

Comparable studies have also shown that low rate of isolation of *Lactobacillus* in female genital tract can be related to vaginal candidiasis[5]. In this study, symptomatic females with candidal

vaginitis had a significantly higher rate of isolation of *C. albicans* and lower rate of *Lactobacillus* than asymptomatic females.such findings were pointers to the health importance of *Lactobacillus* in the vagina.

The experimental *Lactodouche* prepared from the liquid culture of *Lactobacillus* was also quite potent against *C. albicans* and other uropathogens. The preparation can however be produced for commercial purposes by using *Lactobacillus* as starter culture from fermented foods[8,21] such as cassava, *"Ogi", "ogiri", iru* and *kenke*. The innate inhibitory properties of *Lactobacillus* are important in promoting female sexual and reproductive health, besides they are essential in keeping a healthy vaginal environment. Work is on-going on the genetic aspect of *Lactobacillus* as starter culture. It is hoped that more research on *Lactobacillus* and its therapeutic properties will give relief to man and associated health problems.

Acknowledgement

We are grateful to International Foundation for Science for providing fund for research on *Lactobacillus.*

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Original Article

Prevalence of Cotrimoxazole resistant *Streptococcus pneumoniae* and *Haemophilus influenzae* among Nigerian children under five years

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Summary

Health care-givers worldwide are loosing confidence in some of the most useful and affordable antibiotics against *Streptococcus pneumoniae* and *Haemophilus influenzae* which are the most important bacteria pathogens associated with pneumonia. Under Integrated Management of Childhood Illnesses (IMCI), World Health Organization (WHO) recommended the use of cotrimoxazole for first line treatment of pneumonia. Previous study in Nigeria showed that cotrimoxazole is the drug of choice among mothers/ child care-givers for home management of childhood fever. Few data are available on the prevalence of cotrimoxazole resistance of these bacteria among Nigerian children under five years of age. The study determined the prevalence of cotrimoxazole resistance among *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates from Nigerian children with pneumonia.

With informed consent, we recruited into the study 202 children 6-59 months, seen at outpatient department of Massey Street Children's hospital Lagos, who were febrile with difficult breathing /cough. Children on antimicrobial therapy in the previous 2 weeks were excluded. Their blood samples were cultured in Brain-Heart infusion broth and sub-cultured onto blood agar and chocolate agar plates after 24 hours incubation at 37^{o}C. Antimicrobial susceptibility test was carried out according to the National Committee on Clinical Laboratory Standard. Plasmid profiles of multidrug resistant isolates of *Streptococcus pneumoniae* and *Haemophilus influenza*e were demonstrated. The data obtained was analyzed statistically. The age range of the children was 6-59 months and mean age of 22.4 months. Thirty-one (15.3%) blood samples grew *Streptococcus pneumoniae a*nd twenty-six (83.9%) were resistant to cotrimoxazole. Blood samples of 5 (2.5%) patients grew *Haemophilus influenzae* and five (100%) were resistant to cotrimoxazole. Plasmids were demonstrated in 23 (74.2%) of the isolates of *S.pneumoniae* and 4(80%) of *H. influenzae*.The study demonstrated high cotrimoxazole resistance and plasmid carriage by *S. pneumoniae* and *H. influenzae* isolated from the under fives.

Key words: Children under 5 years, cotrimoxazole resistant, *Streptococcus pneumoniae*, *Haemophilus influenzae*

Introduction

Pneumonia is the leading cause of death among under fives, more fatal than AIDS, malaria and measles put together[1,2]. The wide use of cotrimoxazole in treatment of childhood pneumonia in developing countries may be affected by drug resistance which decreases its effectiveness[3]. Pneumonia is the most serious of the lower respiratory infections, even though it can be effectively prevented and treated[4, 5]. More than half of all infant mortality due to pneumonia was recorded in India, Nigeria, Pakistan, Democratic Republic of Congo and Afghanistan[6]. Most deaths due to pneumonia occur in resource limited countries where poor children's immune systems are already weakened by malnutrition and other diseases including malaria, measles and HIV/AIDS[7]. Worldwide about 1.6million under fives die of pneumonia annually and 98% of these infants lived in developing countries[8]. In 2008 it

was estimated that about 177,000 Nigerian children under five years of age died of pneumonia[7]. This places Nigeria first with the highest disease burden in Africa and second in the world[7].

Most lethal aetiology agent of Pneumonia is primarily bacteria which infect the lungs and make breathing difficult[6]. The lungs are made up of small sacs called alveoli usually filled with air when a healthy individual breathes[9].But when a person is infected, the alveoli are filled with pus and fluid making breathing painful and limit oxygen intake[9]. S*reptococcus pneumoniae* and *Haemophilus influenzae* are the most common causes of fatal pneumonia and are responsible for half of infant mortality due to pneumonia[10]. Presently16% to30% of *Streptococcus pneumoniae* globally is multidrug resistant (MDR), which means resistant to ≥ 3 classes of antimicrobial agents[11]. The co-existence of resistance genes with mobile elements such as plasmids, transposons and intergrons facilitates the rapid spread of antibiotic resistance genes among bacteria[12].

Increased resistance to cotrimoxazole (trimethaprim-sulfamethaxazole) in *Streptococcus pneumoniae* and *Haemophilus influenzae* has been reported[13, 14, and 15]. In 1991, WHO published a revised document of Integrated Management of Childhood Illnesses (IMCI) after much experience had been gained with the case management approach and empirical treatment [16]. For management of un-complicated pneumonia at the first level health facility, the document recommended oral cotrimoxazole as the preferred agent [17]. It has been reported that majority of the Nigerian mothers/ child care-givers used cotrimoxazole for home management of childhood fever[18].

The determination of the prevalence of cotrimoxazole resistant *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates from Nigerian under fives with pneumonia becomes imperative because prevention and appropriate treatment of pneumonia could avert numerous deaths due to pneumonia in children every year. This project is aimed to determine the antimicrobial susceptibility pattern and plasmid profile of multidrug resistant species of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolated from Nigerian children with pneumonia

Materials and Methods

Study population: Consent for this study was obtained from parents or child care-givers prior to enrollment, and the study was approved by Nigerian Institute of Medical Research Institutional Review Board (IRB) and the Ethics Committee at Massey Street Children's Hospital, Lagos. We enrolled 202 eligible children with fever and difficult breathing/cough between the ages of 6months – 59months and had not received antimicrobial therapy 2 weeks before presenting to the clinic. Participants were recruited at the out patients department of Massey Street Children's Hospital, Lagos between November 2004 and January 2006.

Specimen collection and bacteria isolation: Two (2)mls of blood was aseptically collected from each participant by the attending clinician and inoculated into corresponding blood culture bottle containing 20mls Brain Heart infusion broth plus 0.05% sodium polyethanolsulfonate (SPS) or liquoid added[to neutralize any bactericidal effect of the blood]. The blood samples were immediately transferred to Microbiology Laboratory in NIMR, Lagos for laboratory investigations.

The blood culture specimens were incubated at 37^{o}C in 10% CO_2 for 24 hours as previously described[19], sub cultured onto blood agar plates made selective for *Streptococcuspneumoniae* by addition of 5µg of gentamicine, and chocolate agar plates supplemented with 5µg of vancomycin for *Haemophilusinfluenzae*[20]. Identification of *Streptococcus pneumoniae* isolates was based on Gram staining, colonial morphology, haemolysis on blood agar, optichin-sensitivity as well as solubility to bile salt [19]. *Haemophilus influenza* was identified based as above in addition to catalase test, oxidase test, X (haemin) and V (NAD) factors dependent, and satilitism[19].

Antimicrobial susceptibility testing:
Antimicrobial susceptibility test was carried out using multidiscs: MASTRING-S (Mast Diagnostics, Merseyside, England) with discs containing antibiotics concentrations specified by the National Committee on Clinical Laboratory Standards (NCCLS) [21] and zone-size interpretative chart according to Kirby-Bauer disc-diffusion method[22]. MDR pathogens were identified based on common MDR phenotypes: resistance to 3 or more antimicrobial classes[11].

Plasmid analysis: Plasmid DNA was isolated from MDR *Streptococcus pneumoniae* species using phenol-chloroform extraction technique as previously described[23].While those from MDR *Haemophilus influenzae* were isolated by modified alkaline lysis *method*[24]. The isolated plasmids were identified using 1.3% horizontal agarose slabs gels in TAE (Tris, Acetate, EDTA) buffer. The electrophoretic tank was used at 70 volts for 1^{1}/2

hour (Sigma chemical Co. ST Louis, USA). Gels were stained with ethidium bromide 0.5µg/ml for 45 minutes and the bands were photographed under ultra violet light[25] using MP4 land camera (Polariod Co. Cambridge, M.A. U.S.A.), equipped with 667 land film and orange written filter (Eastman, Kodak, Co. Rochester N.Y., USA). The molecular weights of the extracted plasmids were determined in relation to mobilities of standard DNA Makers: 1 Kb Plus DNA Ladder (Invitrogen, U.S.A, Cat. No. 10787-018) for plasmids from MDR *Haemophilus influenza* while DNA molecular weight Maker II (0.12-23.1 kbp) from Roche Diagnostics GmbH, Germany Cat. No. 236 250 was used for those from MDR *Streptococcus pneumoniae.***Data analysis: A**ge and sex specific culture positivity rates were analyzed; P- value < 0.05 was regarded as significant. Resistant rates, number and size of plasmids were also calculated.

Results

Two hundred and two children were studied with age range of 6 months to 59 months and average age of 22.4 months. Twenty-three (63.9%) were boys and 13(36.1%) were girls, 36 (17.8%) had culture proven bacterial pneumonia, 31(15.3%) were positive for *Streptococcuspneumoniae*, and 5(2.5%) were positive for *Haemophilus influenzae*. Age was found to be significant (P-value < 0.05) only within the age group 6-12 but sex was not significant (P> 0.05) among any of the age groups (Table1).

Twenty-six isolates (84.0%) of *S.pneumoniae* were resistant to cotrimoxazole, 5(16.0%) were sensitive. The organism also showed high resistance against other classes of antimicrobial agents (Tables 2), although chloramphenicol showed good performance against 28(90.0%) of this organism in-vitro, it can not be recommended for use in-vivo due to previous reports on failure of chloramphenicol therapy in penicillin-resistant *Streptococcuspneumoniae* (PRSP).

Five (100%) of *Haemophilus influenzae* showed resistance to cotrimoxazole. It was found to show 80% resistance to four other classes of the antimicrobial agents tested in vitro. The highest sensitivity of 3(60.0%) of this organism was shown by Cefuroxime (Table 3).

Plasmids were harboured by 23 (99.4%) of *Streptococcuspneumoniae* isolates, their sizes ranged from 1.585 – 2.223 (approximately: 1.6-2.2) kilo base pairs (kbp) and 17 (65.4%) of the bacterial isolates produced more than one plasmids (Table 4). Four (80.0%) of the *Haemophilus influenzae* isolates possessed more than 1 plasmids and sizes 0.726-12.000 (approximately: 0.7-12.0) kbp, 1(20.0%) did not have plasmid DNA (Table 5).

Table 1: Sex and Age distribution of blood culture positive cases of bacterial pneumonia

Age Range (Months)	No of Positive culture (%)	P-value	Sex Female (%)	Male (%)	P-value
6 - 12	15 (41.66)	0.031	5 (33.3)	10 (66.7)	0.262
13- 24	10 (27.77)	0.374	4 (40.0)	6(60.0)	0.531
25-36	9 (25.0)	0.411	4 (44.4)	5 (55.6)	0.701
37-48	1(2.77)	0.645	0 (0.0)	1 (100)	0.912
49-59	1(2.77)	0.645	0 (0.0)	1 (100)	0.912
Total	36(100.0)		13 (36.1)	23(63.9)	

Table 2: In-vitro antimicrobial susceptibility pattern of *Streptococcus pneumoniae* isolates from children under 5 years with pneumonia

Antimicrobial agent	Resistant(%) n =31	Sensitive (%)
Erythromycin	13 (41.9%)	18(58.1%)
Chloramphenicol	3 (10.0%)	28 (90%)
Cotrimoxazole	26 (84.0%)	5 (16%)
Tetracycline	7 (23.1%)	24(76.9%)
Penicillin	18(58.3%)	13 (41.7%)
Cefuroxime	7 (21.4%)	24 (78.6%)
Gentamycin	10(32.1%)	21 (67.9%)
Streptomycin	10(32.1%)	21 (67.9%)

Table 3: *In-vitro* antimicrobial susceptibility pattern of *Haemophilusinfluenzae* isolates from children under 5 years with pneumonia

Antimicrobial agent	Diameter (mm) of standard zones of inhibition indicating *			Resistant n = 5 (%)	Sensitive (%)
	Resistant	Intermediate	Sensitive		
Erythromycin (ERY)	≤13	14-22	≥23	3 (60.0%)	2(40.0%)
Chloramphenicol (CHL)	≤25	26-28	≥29	3 (60.0%)	2 (40.0%)
Cotrimoxazole (COT)	≤10	11-15	≥16	5 (100.0%)	0 (0%)
Tetracycline (TET)	≤14	15-18	≥19	4 (80.0%)	1(20.0%)
Penicillin(PEN)	≤28	21-28	≥29	4(80.0%)	1(20.0%)
Cefuroxime (CEF)	≤28	-	≥29	2 (40.0%)	3 (60.0%)
Gentamycin (GEN)	≤12	-	≥13	4(80.0%)	1 (20.0%)
Streptomycin (STR)	≤11	12-14	≥15	4(80.0%)	1 (20.0%)

*Inhibition zone diameter (IZD) breakpoint for antibiotics tested according toNational Committee on Clinical Laboratory Standard (1988).

Table 4: Plasmid carriage and distribution among multiple drug resistant *Streptococcus pneumoniae* isolates

Streptococcus pneumoniae isolates (%) n = 26	Plasmids isolated	Molecular weight (kilo base pairs)
3 (11.5)	0	0
6 (23.1)	1	1.6
4 (15.4)	2	1.8, 1.8
13 (50.0)	3	2.2, 2.2, 2.2

Table 5: Plasmid carriage and distribution among multiple drug resistant *Haemophilus influenzae* isolates

Haemophilus influenzae Isolatesn = 5(%)	Plasmids isolated	Molecular weight (kilo base pairs)
1 (20.0%)	0	0
1 (20.0%)	2	0.7, 0.7
1 (20.0%)	3	1.1, 1.1, 1.1
1 (20.0%)	4	2.0, 2.0, 2.0, 2.0
1 (20.0%)	5	12.0, 12.0, 12.0, 12.0, 12.0

Discussion

Earlier studies in Australia Ghana showed a prevalence of 6% and 9.2% respectively compared to 17.8% in this study[26,27]. This difference may be due to the fact that our participants in this study were on antibiotics before presenting to the clinic. The present study showed most culture positive pneumonia cases 15(41.7%) to be within 6-12 months of age (P< 0.05) and it is in agreement with WHO (2009) report which showed that pneumonia mostly affects children < 2 years[10].

In this study the significantly higher proportion of boys 23(63.9%) compared to 13(36.1) were girls (P<0.05) is similar to earlier findings by Oyejide and Osunsi which recorded higher incidence of pneumonia among boys compared girls[28]. We also recorded 31(15.3%) of the children to have positive culture for *Streptococcus pneumoniae* similar to 16.4% reported by Walsh *and colleagues in* Malawi[29]. However the finding in our study of 2.5% 5(5) of the participants were culture positive for *Haemophilusinfluenzae* did not correlate with the report of Lepage and colleagues in Rwanda which reported only 0.3%[30]. A possible reason for this difference could be due to the fact that the children in our study were significantly younger (22.4 months) compared to a mean of 75months in the Rwanda study.

We found 26(83.9%) of *Streptococcus pneumoniae* and 5(100%) of *Haemophilusinfluenzae* isolates resistant to cotrimoxazole. The finding is similar to with the reports from United Arab Emirates[31], but not in agreement with reports from Zambia[32]. While our study and UAE study utilized blood samples, the Zambia study used nasopharygeal specimens.

The percentage of multidrug resistant bacterial isolates in our study than contained plasmids averages 80% for *Streptococcuspneumoniae*(82.1%) and *Haemophilus influenzae* (80.0%) compared to 12.5% reported by by Chalkley and Koornhof in South Africa[33] for *Streptococcuspneumoniae*and 38.5% reported by Campos and colleague in Spain for *Haemophilus influenzae* isolates

Conclusion

The study demonstrated high cotrimoxazole resistance and plasmid carriage by the *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates from the under 5 childrenThere is a need for the appropriate authority to put in place ongoing surveillance to monitor trends of cotrimoxazole resistance of these bacteria among children < 5years in various geo-political zones of Nigeria.

Further studies to determine serotypes of these *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates should be carried out which will show the local serotypes of these organisms in Nigeria to ensure use of effective antimicrobial agents for the management of pediatric pneumonia.

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Ethics Corner

The Fate of "Valuable" Data Obtained Through Unethical Means

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Introduction

HUMAN experimentation since World War II has created some difficult problems with the increasing employment of patients as experimental subjects when it was apparent that they would not have been available if they had been truly aware of the uses that would be made of them. Evidence is at hand that many of the patients never had the risk satisfactorily explained to them, and it seems obvious that further hundreds have not known that they were the subjects of an experiment and grave consequences have been suffered as a direct result of the experiments.

This treatise however attempt to discuss the position of 'ethics' on using data obtained from such research involving human subject without prior ethical consideration, the different schools of thoughts on the subject matter and comparing the best of both worlds… what it is or what it ought to be?

Case Study of Nazi Experiment: Nuremberg Trials: Following World War II, International Military Tribunal at Nuremberg was set up against leading Nazi doctors who were charged with war crimes and crimes against humanity. And they were found guilty of premeditated murder masqueraded as research.

Mostly this has been the experience nowadays among many erring scientist involved in biomedical research who succumb easily to publishing papers by all means possible as they always say "Publish or Perish". These scientists consider ethical consideration and or ethical review for human participant protection as a form of delay or slowing down the rate of completing their research work on time and speedy publishing without considering the human participants. It is however imperative to realize the implication of such acts in modern day science where the rights of participants are considered paramount than the biomedical science research conduct itself.

The nature of the experiments conducted by the Nazi doctors was as stated below;

1. Persons were forced to become subjects in very dangerous studies against their will,
2. Nearly all subjects endured incredible suffering, mutilation, and indescribable pain; and
3. The experiments often were deliberately designed to terminate in a fatal outcome for their victims.
4. No ethical consideration before the conduct of the research
5. Experiments designed to gain knowledge on certain wartime conditions
6. The Nazi doctors considered "military necessity" adequate justification for their heinous experiments.
7. They justified their acts by saying that the prisoners were condemned to death anyway e.g. Pfizer Trovan study in Kano, Nigeria (Adejumo, 2008), hence no need for their consent.

There were three basic categories of experiments-namely (1) Medico-Military Research; (2) Miscellaneous, Ad Hoc Experiments; and (3) Anthropological/Racial Motivated reasons. In the latter, prisoners were killed to assemble a collection of skeletons for "anthropological investigations". Those killed were considered

prototypes of "repulsive but characteristic sub-human" There were different types of experiments performed on their subjects such as High-Altitude (low pressure) experiments, Freezing experiments followed by re-warming, Sea-Water experiments, Malaria experiments, Mustard Gas experiments, Tuberculosis experiments, Sterilization experiments for prisoners – chemical and X-ray sterilization, Poison experiments – How quickly to execute Russian soldiers and sulfanilamide experiment among others

However, there were various important scientific results obtained from these heinous experiments masqueraded as research. Thus, giving rise to serious ethical concern in using tainted data from experiments on patients who were murdered and tortured by the Nazis in the name of "research." In considering the data we should also Imagine the extreme feeling of discomfort, and the mortified look of horror upon the family of the murdered 'subjects'. Secondly, any analysis that fails to see realistically the Nazi data as a blood soaked document fails to comprehend fully the magnitude of the issue. The schools of thought are;

- Proposed use of Nazi scientific data
- Banning the use of Nazi scientific data
- The best of both worlds…… thinking of accomplice law

Proposed Use of Nazi Scientific Data: Doctor Robert Pozos – is a Director at the Hypothermia Laboratory -University of Minnesota, college of Medicine at Duluth. His research is devoted to methods of re-warming frozen victims of cold. Much of what he and other hypothermia specialists know about rescuing frozen victims is the result of trial and error performed in hospital emergency rooms. Pozos believes that many of the existing re-warming techniques that have been used thus far lack a certain amount of critical ethical and scientific thinking. This is because ethically one cannot allow the subject's temperature to drop below 36^0C. So, Pozos had to speculate what the effects would be on a human being at lower temperatures. In aberrant, the only experiment that put humans through extensive hypothermia research (at lower temperatures) was the Nazis research experiments.

The realities were that data morally tainted and soaked with blood does exist till today and there is dreaded possibility that perhaps the Nazi doctors actually learned something that today could help save lives or "benefit" society. Rebutting the notion of moral complicity – We should be willing to separate the evil of the original act from the good intentions of any contemporary work. If the data is valid it cannot be invalidated no matter how objectionable we may find the unethical behavior used to obtain them. Data that are unobtainable using today's much more rigorous ethical standards may be of special significance and ensuring that such unethical concepts do not happen again will be achieved by an understanding of ethical principles and not by a refusal to utilize any valid data from those earlier studies

Other concerns are that Is it appropriate to pass judgment(s) on behalf of the victims of the Nazi experiments? Would the victims have approved of our analysis and conclusions? Would they be consoled to learn that their deaths produced life, or would they be mortified to know that their suffering is being exploited by others? This is a subject of ethical discussion.

Reasons Not To Use The Data: There are various reasons why the use of Nazi's data is considered unacceptable ethically;

1. Showing Respect to those killed under the disguise of research through unethical means
2. Moral Complicity argument- as explained below
    i. Citing the data implicates us to the atrocities
    ii. We become perpetrators of original crime once we succumb to the outcome
    iii. Our motives today cannot be isolated from the manner in which the research was conducted once we agree to cite the data
    iv. To cite unethical data is to validate it
    v. To stop the continuing trend of such bastardized act we should completely dissociate ourselves from the data or else many scientists will be citing the unethical precedence to perpetrate their selfish unethical acts. So we should be careful not to institutionalize what we condemn.

Justification/Comparison of current spate of research without ethical consideration with Nazis experiment: From all the above, comparison the Nazi's experiments with today's' attitude of researchers, we discovered that attitudes of the Nazis doctor are not so very far removed from the attitudes of modern days scientists -So much scientific and ethical misconduct!. Therefore all the unethical attitudes need to be explored and rejected, and a

transformation of attitudes is only possible by refusal to have anything to do with work that emanated from such behavior. The medical /research profession should strive to maintain its integrity and the confidence of the public. It should not operate with tarnished reputations.The relics of the memory of Nazis experiment cannot be waived; hence the data there from cannot be used

Considering the 'best of both sides": In consideration of the best of both sides: some important thought provoking questions will require urgent and convincing answers. Questions such as

i) What if the information has a potential of saving lives?
ii) Won't justice be ultimately served if we are to allow life to emerge from the Nazi murders?
iii) What about the accomplice law?

This means the best of both worlds will entail what we refer to as a 'Doctrine of Double Effect' which could infer that we can use the data under the following circumstances or if all the conditions below are fulfilled;

i) There are NO alternatives
ii) The data is clearly scientifically valid
iii) Accomplice law is abolished
iv) Potential high benefits offset the risks
v) Condemn the Nazis horrific experiments by disclaimer, at the same time use the data for potential benefit(s) - [Doctrine of Double Effect].

Beecher's Analogy – The Exclusionary Rule: Similarly, Dr. Henry Beecher, the late Harvard Medical School Professor, analogized the use of the Nazi data to the inadmissibility of unconstitutionally obtained evidence. Dr. Beecher said that even though suppression of the data would constitute a loss to medicine in a specific localized sense: this loss, it seems, would be less important than the far reaching moral loss to medicine if the data were to be published (Beecher, 1966) .Beecher's analogy is to be given serious consideration. Although use of the Nazi data might benefit some lives, a larger bioethical problem arises. By conferring a scientific martyrdom on the victims, it would tend to make them our retrospective guinea pigs, and we, their retrospective torturers.

Conclusion

Oneschool of thought believes absolute censorship of the Nazi data does not seem proper, especially when the secrets of saving lives may lie solely in its contents. Society must decide on its use by correctly understanding the exact benefits to be gained. When the value of the Nazi data is of great value to humanity, then the morally appropriate policy would be to utilize the data, while explicitly condemning the atrocities. But the data should not be used just with a single disclaimer. To further justify its use, the scientific validity of the experiment must be clear; there must be no other alternative source from which to gain that information, and the tenacity to save lives must be evident.

Once a decision to use the data has been made, experts suggest that it must not be included as ordinary scientific research, just to be cited and placed in a medical journal. The citation of the data must contain a thorough expose of exactly what tortures and atrocities were committed for that experiment. Citations of the Nazi data must be accompanied with the author's condemnation of the data as a lesson in horror and as a moral aberration in medical science. The author who chooses to use the Nazi data must be prepared to expose the Nazi doctors' immoral experiments as medical evil, never to be repeated.

This implies that all research conduct without ethical approval and or ethics review satisfying all the benchmarks that makes biomedical research ethical as stated by Emanuel et al., (2004) is considered unethical and heinous and hence data generated therefrom is not admissible and or acceptable in the scientific world.

Finally, in this age of so many medical advances, a humanistic perspective is hoped for. The process of critically examining past history will hopefully prompt a greater effort to curb potential human participant abuse. Therefore as a result of retrospective moral judgment ethical principles and guidelines have evolved coupled with serious efforts to hold medical researchers accountable for any wrong doing. By so doing, we hope there will be better protection for human research participants.

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Eratum

We recieved complaints from Mrs. Outonye – the corresponding author, that in their original research article titled **Sexual Transmitted Infections and HIV among female commercial sex workers in Lagos Nigeria** published in the Jan 2011;vol 6 edition of Nigerian Journal of Clinical and Biomedical Research), the authors were wrongly listed as **Otuonye NM, Onwuatuelo IR, Onwuamah CK, Okwuzu JO, Adeneye AK, Oparaugo CT, Adesesan AA**, **Akintunde GB, Ohiku FO, Uwandu M Fowora MA.**

We have checked and confirmed that her observation was correct and is as a result of error during the publication process.

The correct title, authors and their affiliations in the published article are as shown below

Sexual Transmitted Infections and HIV among female commercial sex workers in Lagos Nigeria

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The first page of the article with correct listing of authors and their affiliationsis reproduced in the next page.

Nigerian Journal of Clinical & Biomedical Research (Jan. 2011) Vol. 5 No.1, 73 – 80

Original Research Article

Sexual Transmitted Infections and HIV among female commercial sex workers in Lagos Nigeria

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Keywords: Female Commercial Sex workers, HIV, STIs, anal sex, oral sex, contraceptives

SUMMARY

Sexually transmitted infections (STIs) remain a public health problem of major significance in most part of the world and Female Sex Workers (FSW), are generally considered to be at risk of STIs/HIV infections. Four hundred and fifty FSW (15-35years) were randomly selected from three local government areas in Lagos State to participate in the study. They had Pre and Post-test HIV counseling, completed a questionnaire to provide information on demography, sexual behavior (anal and oral), use of contraceptives and douching. Cervical Swabs were used to screen for sexually transmitted pathogens (standard methods) and to detect Chlamydia antigen (Quick-Vue Chlamydia kit). Wet preparations were made from HVS to detect yeasts and Trichomonads. Blood samples were screened for antibodies to HIV (ELISA and Western blot) and syphilis (RPR - Rapid Plasma Reagin test kit andTPHA – Treponema Heamagglutination test kit). Chi square analysis was used to test the significance between HIV/STIs infections and sexual behavior, contraceptive use and douching.Pathogens identified were Candida spp. (32.5%), HIV-1 (31.0%), Trichomonas (23.2%) and bacteria (13.3%). STIs/HIV infections were highest (64.5%) in the age group 26-30 years. Among FSW who douched, who had anal sex and those who had oral sex, the prevalence of HIV/STIs was 81.1%, 75.0% and 67.5%.Vaginal douching, anal and oral sex practices suggest increased susceptibility to HIV/STIs. Appropriate sex education programmes on these practices and advocacy on the need to access available health programmes is needed to reduce the burden of STIs/HIV in FSW in Lagos Nigeria.

INTRODUCTION

Globally, female Sex workers are generally considered to be at high risk of STIs/HIV infections due to the large numbers of short term sexual partners and other high risk behaviours they are involved in, such as low condom use, alcohol and drug abuse [1]. They also lack sufficient information and understanding of HIV/AIDS/STIs, their vulnerability to it, how to prevent it, and the self-confidence necessary to protect themselves[2]. Some young people become prostitutes in order to make money[3]. Similarly, economic deprivation leads many young women in sub-Sahara Africa into sexual relationships with older men - sometimes known as 'sugar daddies' that provide money and other necessities, such as clothing and school fees in exchange for sex[3]. Sexual activity involving persons below 18 years old nearly always involve adult accomplices including parents and older siblings. Also, child sexual workers are victims of sexual abuse. These children often see themselves as their only supporter, under such circumstances, some fall into Sexual work as a way to survive[4]. Often, they become involved in the criminal-justice system as offenders, or to escape the life that they have come to lead, they get involved in the use and/or sale of drugs, theft, or robberies[5]. UNICEF (1997) estimates that more than a hundred million children worldwide are employed as "sex workers," subjects of pornography, or both. In addition, hundreds of thousands of children shuttle the planet each year as part of a well-concealed network operated by international traffickers in children for sex[3].

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ACKNOWLEDGEMENTS

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