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EVALUATION OF USEFULNESS OF POLYMERASE CHAIN REACTION IN THE DIAGNOSIS OF MALARIA IN NIGERIA

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Abstract

Microscopy has been the most common technique for the diagnosis of malaria in south western Nigeria. This study was undertaken to determine the efficiency of PCR for malaria diagnosis in south western Nigeria. A total of 450 samples submitted for malaria diagnosis at Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife between the months of January and December, 2009 were used. Methods used included Giemsa staining procedure for estimation of parasite densities and polymerase chain reaction (PCR) to detect the presence of malaria parasite in the whole blood. Using microscopy as reference gold standard, patients comprising 120 males and 330 females with age ranging between less than 1 and 60 samples were used. In all, about 255 (56.7 %) of the samples were positive for microscopy, while 75 (16.7 %) with high parasitaemia on microscopy were positive for PCR analysis. The study concluded that PCR for diagnosis of malaria has sensitivity of 29.4% and specificity of 100% using crude method of DNA extraction while the use of DNA extraction kit has sensitivity of 90.2% and specificity of 100%, hence effort should be geared towards increasing the sensitivity and reduce the cost of doing the test in low resource country like Nigeria.

Running Title: **Polymerase Chain Reaction in the Diagnosis of Malaria**

Key Words: **Plasmodium; Polymerase chain reaction; Microscopy; parasitaemia**

INTRODUCTION

Malaria is an infection caused by the malaria parasite entering the blood stream (1). The parasite may gain entry through the bite of an infected mosquito or infected blood transferred by blood transfusion or a contaminated injection needle. Malaria is the most important tropical disease, remaining widespread throughout the tropics, but also occurring in many temperate regions (2). It exacts a heavy toll of illness and death especially among children and pregnant women. It also posses a risk to travellers and immigrants with imported cases increasing in non-endemic areas (2). There was an estimated 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly a million deaths, mostly of children under 5 years. About 109 countries were endemic for malaria in 2008, 45 within the WHO African Region (3, 4).

Malaria presents a diagnostic challenge to laboratories in most countries. Microscopic examination of Giemsa-stained thick and thin blood smears has been identified as the most common technique to diagnose malaria since last 100 years (5, 6). Microscopy continues to be the gold standard for identification of

Plasmodium spp. in the laboratory setting (7, 8). Although easy to apply and cost-effective, this technique assumes that laboratories have certain infrastructure in place with highly skilled professionals and in detecting low level parasitaemia - 10 to 50 parasites/µl, so the sensitivity may fluctuate depending upon the skill of technician (9).

WHO has recognized the urgent need for simple and cost-effective diagnostic tests for malaria to overcome the deficiencies of both light microscopy and clinical diagnosis. To overcome such limitations, several methods have been in use including the staining of parasite DNA and RNA with acridine orange: the quantitative buffy coat method (QBC); methods based on the detection of the enzyme lactate dehydrogenase (pLDH), rapid antigen capture assay that detects circulating *P. falciparum* histidine rich protein-2 (PfHRP-II) (10), used mainly in diagnosing malaria in non-

immune individuals or in epidemiologic studies.

Polymerase chain reaction (PCR) based assays have been used mainly for the assessment of the sensitivity and specificity of microscopy and rapid diagnostic tests

(RDTs) and may be of clinical value in some selected situations (11, 12). PCR based tests have shown remarkable capacity to detect malarial parasites in mixed infections and low parasite count and are also sensitive when compared to microscopic examination (13, 14). It has been estimated that PCR can detect malaria infections with parasitemia as low as 5 parasites/µl (0.0001% parasitaemia) (15).

The value of PCR as a means of malaria parasite diagnostic technique lies in its sensitivity, its capability of identifying malaria parasites to the species level, as well as its ability to detect five parasites or less/µl of blood (16). The aim of this study was to apply the technique of polymerase chain reaction (PCR) in the diagnosis of malaria parasite in a community with limited resources and infrastructural facilities, and compare its sensitivity and specificity with microscopy - a gold standard. This will enable us to appraise the use of molecular technique in the diagnosis of malaria in order to improve the laboratory and clinical diagnosis of this disease.

MATERIALS AND METHODS

Sample collection

A total number of 450 blood samples submitted for blood film in diagnostic laboratory at Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife between the months of January and December, 2009 were used. These blood samples were collected in EDTA bottles and transported in an ice pack to the Molecular Biology Laboratory of Biomedical Sciences Department, Ladoke Akintola University of Technology, Osogbo Campus.

Thick and thin blood films

Thick and thin blood films were prepared according to the methods of (17). Air-dried thick and absolute methanol-fixed thin films were stained with 10% Giemsa solution for 10 minute. Malaria parasites were examined using oil immersion lens objective (×100) of a high quality microscope (Olympus) with an incandescent light source.

Parasite density

Thick and thin films were examined microscopically. Each species of *Plasmodium* as well as parasite densities in 100% was identified. The parasite density was counted against 500 leukocytes and was then expressed as the number of trophozoite per microlitre by assuming leukocyte count of 7000 per microlitre (18).

DNA extraction

***Kit method*:** Two hundred microlitres (200 µl) each from all the samples (450) was subjected to DNA extraction from whole blood collected in EDTA anticoagulant bottle using Ultra Blood Spin kit (Carlsbad, Germany), according to manufacturer's instructions.

Crude method: A modified method of extraction was carried out using digestion buffer. Two hundred microlitres (200 µl) portion of the blood sample was dispensed in 1.5 ml centrifuge tubes, and the tubes centrifuged at 2,000 g for 5 minutes at room temperature in order to remove the plasma containing proteins. The packed red cell was washed twice in 1 ml distilled water; after which 100 µl of digestion buffer was added by pipetting up and down with a micropipette. The mixture was incubated at 60^{o}C for 1 hour with occasional mixing, the digest was boiled at boiling temperature (100^{o}C) for 10 minutes, and was snap cooled on ice. The mixture was centrifuged at 12,000 g for 1 minute. The supernatant (DNA) was separated into a new tube for storage in order to prevent inhibition by the sediment which can still contain haemoglobin (19).

Optimization of PCR

Optimization was done in order to know the desired amplification condition for a small subunit ribosomal RNA (ssrRNA) of size 1.7 kb. PCR master mix was prepared to allow for 20 µl working volume. For a typical 5 reactions, the master mix contained 55 µl of PCR water, 10 µl of the forward primer (GGA TAA GTA CGG AAA AGC TGT AGC), 10 µl reverse primer (CGA CTT CTC CTT CCT TTA AAA GAT), and 20 µl of the Taq mix (contains the dNTPs and polymerase). Nineteen microlitres (19 µl) of the master mix was aliquoted into each of the PCR tubes and 1 µl of the extracted DNA sample was added with positive and negative controls inclusive. The amplification condition was at 1 min denaturation at 94 ^{o}C, 1 min reannealing at 56 ^{o}C, and 3 min extension at 72^{o}C, for a total of 35 cycles. Maximum care was taken to avoid contamination by ensuring strict physical barrier and working in a well sectored laboratory of high hygiene.

***PCR for the detection of Plasmodium* species**

PCR master mix was prepared to allow for 30 µl working volume. For a typical 30 reactions, the master mix contained 512 µl of PCR water, 96 µl of the forward primer (GGA TAA GTA CGG AAA AGC TGT AGC), 96 µl of reverse primer (CGA CTT CTC CTT CCT TTA AAA GAT), and 192 µl of Taq mix (0.2 mM dNTPs; 1.5 mM $MgCl_2$; 50 mM KCl; 25 units/ml *Taq* DNA polymerase), and amplification carried out in a thermal cycler, the master mix contained a final concentration of 1 µM each for forward primer and backward primer, 28 µl of the master mix was aliquoted into PCR tubes and 2 µl of the extracted DNA sample was added and mixed. The amplification condition was at 1 min denaturation at 94°C, 1 min annealing at 56°C, and 3 min extension at 72°C, for a total of 35 cycles (Mathiopoulos et al., 1993) using GeneAmp PCR System 9700.

Agarose gel electrophoresis

Agarose gel electrophoresis was carried out using 1 % agarose gel in 0.5 X Tris borate EDTA buffer (44.5 mM Tris borate and 1 mM EDTA, pH 8.3), agarose was weighed into a clean conical flask containing 95 ml of distilled water, which was made up to 100 ml by addition of 5 ml of Tris borate EDTA buffer. Dissolved agarose was heated briefly for effective dissolution in microwave oven, it was allowed to cool to about 50 °C, ethidium bromide was added. The edge of a clean dry Perspex plate (7 x 7) was tappered with paper tape, molten agarose was poured into a dry Perspex plate sealed with paper tape to form a mould and with inserted combs to create wells for loading the PCR products and allowed to set (20-30 min at room temperature). Combs and paper tapes were removed and gel in the tray was carefully placed in the electrophoretic tank containing electrophoresis buffer. The amplified product was loaded into the agarose in the tank. To load, the DNA (10 µl) was mixed with 1 µl of loading dye (bromophenol blue, xylene cyanole, sucrose) on a Para film "M" paper and was loaded into the agarose well using micropipette. DNA ladder was also loaded to one end of the gel using a micropipette. Electrophoresis was done at 70 Volts for 90 min. The DNA bands were visualised with short wave ultraviolet transilluminator and photograph taken using a Syngene gel documentation system (Syngene, UK). The sizes of the DNA bands were estimated from plots of the relative mobilities against the DNA ladder reference.

Data Analysis.

Data were analysed using statistical package within the Microsoft Excel and Epi-info software for Disease control and prevention, USA. Chi square was used to determine the effect of DNA extraction methods on the data obtained. The p value less than 0.05 was considered to be significant.

RESULTS

On optimisation, amplification was performed in a thermal cycler with a three-step cycling programme: 1 minute denaturation at 94°C followed by 1 min annealing at 56°C and a 3 min extension at 72°C for a total of 35 cycles was successful in respect to both positive and negative control, and amplification of the 1.7 kb product was visible at this condition, and this dictated the condition for overall amplification. Representatives of the PCR products sent for sequencing confirmed the product to be DNA template for ssrRNA. The result of PCR showed specific bands of 1.7 kb (Figure 1), 230 of the 450 samples (51.0%) were positive by PCR. Of this, 92 were males and 138 were females. A low proportion, 25 (9.8%) of all microscopy positive samples were negative by PCR and 230 (100%) of PCR positive samples were positive for microscopy (Figure 2).

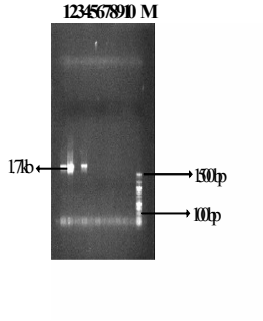

FIG. 1

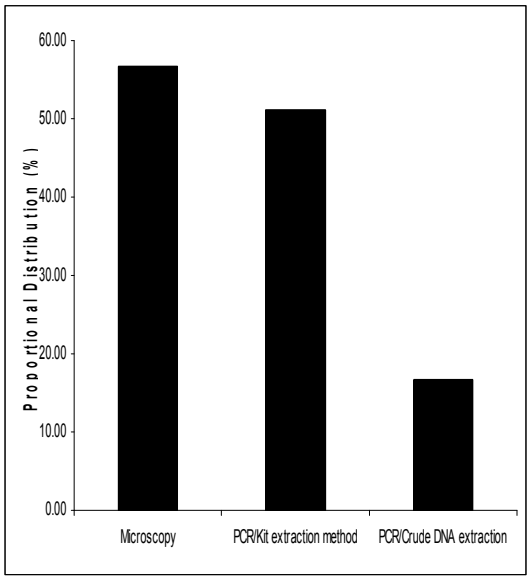

FIG. 2

Figure 1 . PCR detection of *Plasmodium* species. Lane 1: positive control; Lanes 2-9: samples; Lane 10: Negative control; Lane M: 100 bp DNA ladder size marker. Arrow indicating the expected 1.7 kb DNA fragments

Figure 2: Distribution of malaria parasite according to technique employed

On the basis of repeatedly negative malaria smears and PCR negative results, 180 patients did not have malaria. The overall sensitivity, specificity, PPV, and NPV of the PCR test were found to be 90.2%, 100%, 100%, and 87.8%, respectively. A total prevalence of 56.7 % was obtained using microscopy while prevalence of 51.1% using PCR where DNA extraction kit was used (Figure 2). We also noticed significant difference (X^2 = 117.62; $p < 0.01$) in the performance of PCR with respect to DNA extraction method adopted – kit versus crude method; kit DNA extraction method produced results very close to microscopy (51.1%) while crude method efficiency of detecting malaria parasite was very low (16.7% prevalence, Fig. 2).

A total of 450 samples were used in this study. The age was between 1 and 60 years (mean = 33.4; median = 29.5). Blood film revealed that 255 (56.7 %) had malaria parasite. Seventy five (62.5%) males out of 120 had malaria parasites by blood film while 180 (54.6%) females were blood film positive (Fig. 3).

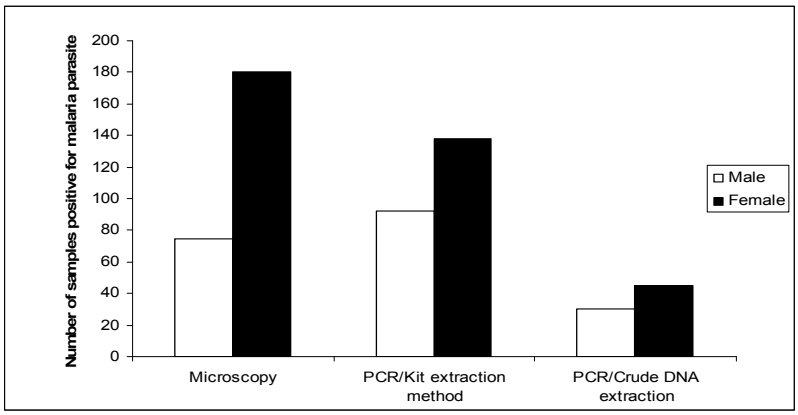

FIG. 3

Figure 3. Sex prevalence of malaria parasite according to the technique

A panel of 144 samples that had non-concordant results were retested and the performance of each assay calculated by comparing the results from the second tests with those of the initial test (i.e. initial test was used as the reference standard for statistical purposes). The data from this test clearly demonstrated that PCR was more repeatable, less subjective test than microscopy with parasite densities of more than 50 parasites/µl. Furthermore, the samples that were mistaken to be negative in PCR were found to have low parasite counts. The study also noticed that the crude method provided DNA sample that were PCR inhibited as a result of presence of haemoglobin, while the use of DNA extraction kit ameliorated this condition.

DISCUSSION

In this study, microscopic examination using Giemsa stain showed specific and defined malaria parasites in blood smears. Microscopic examination of Giemsa-stained thick and thin blood smears has been the diagnostic method of choice for species identification in epidemiologic studies and medical diagnosis (20). The method is simple, does not require highly equipped facilities, and in most cases enables differentiation among the four species causing malaria in humans when performed by experienced personnel. However, microscopy is often time-consuming and laborious, and it is estimated that even a skilled person can evaluate only 60 to 80 specimens per day under field conditions (21). The recommended method and current gold standard used for the routine laboratory diagnosis of malaria is the microscopic examination of stained thick and thin blood films, particularly with the additional sensitivity offered by examination of thick blood films (22).

PCR based methods have been consistently shown to be powerful tool for malaria diagnosis (23, 24). There was extremely poor concordance between microscopy and PCR at relatively low parasite densities. When PCR was considered the reference standard, the performance of microscopy was just as poor as that observed for PCR when microscopy was used as the reference standard. Because it was difficult to determine whether microscopy or PCR was the more accurate assay, all non-concordant samples were retested in order to determine which method was the more repeatable test (assuming that the more repeatable assay was the more accurate). The data from this test clearly demonstrated that PCR was more repeatable. PCR presented with a good performance criteria in terms of reproducibility when compared to microscopy, coupled with 100 % specificity, 90.9% % sensitivity, 100 % positive predictive value (PPV), and 87.8 % negative predictive value (NPV) PCR values, the specificity and PPV can be said to be in agreement with a study carried out by Barker et al. (25), and Speers et al. (26) with 100% specificity. A study from an endemic area of Brazil reported a sensitivity of

PCR to be 73% using blood stained in filter paper strips in condition of low level parasitaemia (27). However, another study has reported an overall sensitivity of 97.4% for PCR compared to microscopy (28), result of 90.9% sensitivity (low), and 87.8 % negative predictive value (high) obtained from this study is not in agreement with the cited studies and this could be as a result of degradation of the target DNA after extraction with repeated freezing and thawing in the presence of *Plasmodium* species sequences that lack sequence recognised by the used primers or technical faults while DNA storage may give false negative results (25) especially when amplifying a large DNA fragment such as 1.7 kb. The repeated freezing and thawing of the samples due to electricity cuts/load shedding might have also contributed to the degradation of the DNA in the samples (29).

In improving sensitivity, it can be recommended that method of extraction should be well monitored in order to prevent PCR internal inhibitors to reduce false negative results. Example of which is haemoglobin in blood which had been shown by this study not to be well cleaned off from the extracted DNA by the crude method of extraction, this was confirmed due to internal inhibition of some samples which were negative for PCR, but were positive with DNA extracted by kit, thereby given kit method of extraction a higher recommendation. Also, investigating smaller amplicon (206 bp, 240 bp, 620 bp) by targeting small subunit ribosomal RNA (ssrRNA) gene can aid more sensitivity as seen in most studies (30, 31) rather than bigger fragment as done for this study.

In spite of having a good Microscopist, this study highlights the difficulty in conducting an active surveillance programme in areas where infection rates and parasite densities are low. Although, sensitivity of PCR can be related to parasite density (30, 31), data here indicates that PCR can be a viable method for conducting active malaria surveillance in south western Nigeria, if sensitivity is increased.

In conclusion, most studies have found that PCR-based tests have shown remarkable capacity to detect malaria parasites in low parasite count, and are also more sensitive when compared to microscopic examination (13, 27). The study concluded that PCR has sensitivity of 90.9 % and specificity of 100 % in malarial infection diagnosis. With this, PCR could be a good tool in confirming the clinically strongly suspected but microscopically negative cases of malaria infection, but effort should be made to increase the sensitivity of PCR to 100% in Nigeria.

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REFERENCES

1. Longworth DL (1995): Drug resistant malaria in children and in travellers. *Paediatric Clinics of North America* 42(3): 649-664.

2. World Health Organization (2001): WHO/MAL/2000.1091. New perspectives in malaria diagnosis. *World Health Organization*, Geneva, Switzerland.

3. Winstanley P, Ward S, Snow R *et al.* (2004): Therapy of falciparum malaria in Sub-Saharan Africa: from molecule to policy. *Clin Microbiol Rev* 17: 612-37.

4. World Health Organization (2008): World Malaria Report, Geneva, Switzerland.

5. WHO/CDS/RBM. New perspectives malaria diagnosis. Report of a joint WHO/ USAID informal consultation, WHO, Geneva, 2000.

6. Rougemont M, Saanen MV, Sahli R *et al.* (2004): Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *J Clin Microbiol* 42: 5636–43.

7. Makler MT, Palmer CJ, Ager AL (1998): A review of practical techniques for the diagnosis of malaria. *Ann Trop Med Parasitol* 92: 419–33.

8. Ndao M, Bandyayera E, Kokoskin E *et al.* (2004): Comparison of blood smear,

antigen detection, and nested-PCR methods for screening refugees from regions where malaria is endemic after a malaria outbreak in Quebec, Canada. *J Clin Microbiol* 42: 2694–700.

9. Payne D (1998): Use and limitations of light microscopy for diagnosing malaria at the primary health care level. *Bull WHO* 66: 621-6.

10. Ghimire P, Samantaray JC, Mirdha BR (2001): Comparative evaluation of four different techniques used in the diagnosis of malaria. *J Inst Med* (Nepal) 23: 50–4.

11. Hanscheid T, Grobusch MP (2002): How useful is PCR in the diagnosis of malaria? *Trends Parasitol* 18: 395-8.

12. Bell D, Peeling RW (2006): Evaluation of rapid diagnostic tests: malaria. *Nat Rev Microbiol* 4: 34-8.

13. Brown AE, Kain CK, Pipithkul J *et al.* (1992): Demonstration by the polymerase chain reaction of mixed *Plasmodium falciparum* and *Plasmodium vivax* undetected by conventional microscopy. *Trans Roy Soc Trop Med Hyg* 86: 609-12.

14. Zakeri S, Najafabadi ST, Zare A *et al.* ((2002): Detection of malaria parasites by nested PCR in south-eastern, Iran: evidence of highly mixed infections in Ghahbahar District. *Malaria J* 1: 2-8.

15. Zalis MG, Ferreira-da-Cruz MF, Balthazar-Guedes HC *et al.* (1996): Malaria diagnosis: standardization of a polymerase chain reaction for the detection of *Plasmodium falciparum* parasites in individuals with low-grade parasitemia. *Parasitol Res* 82: 612–6.

16. Snounou G, Viriyakosol S, Jarra W, Thaithong S, and Brown KN. (1993): Identification of the four human malarial species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol. Biochem. Parasitol.* 58: 283–292.

17. Cheesbrough M. (2005): District Laboratory Practice in Tropical Countries. Low price ed. *Cambridge University Press* Part 2: pg 244-248.

18. Kain KC, Brown AE, Mirabelli L, and Webster HK (1993): Detection of *Plasmodium vivax* by polymerase chain reaction in a field study. *J. Infect. Dis* 1993; 168: 1323–1326.

19. Kitada k, Oka S, Kojin T, Kimura S, and Nakamura Y (1991): Diagnosis of *Pneumocystis carinii* pneumonia by 5S ribosomal DNA amplification. *J. Protozool.* 38 (Suppl): 90S-91S.

20. Warhurst DC and Williams JE (1996): Laboratory diagnosis of malaria. *J. Clin. Pathol.* 49: 533–538.

21. Gardner MJ, Hall N, Fung E, et al. (2002): Genome sequence of the human malaria parasite *P. falciparum*. *Nature* 419: 498–511.

22. Moody A. (2002): Rapid Diagnostic Tests for Malaria Parasites. *Rev. Clin. Microbiol* 15: 166-178.

23. Di Santi SM, Kirchgatter K, Brunialti KCS, et al. (2004): PCR–based diagnosis to evaluate the performance of malaria reference centers. *Rev. Inst. Med. Trop.* 46: 183-7.

24. Coleman RE, Sattabongkot J, Promstaporm S et al. (2006): Comparison of PCR and microscopy for the detection of asymptomatic malaria in a *Plasmodium falciparum/vivax* endemic area in Thailandm. *Malaria J* 5: 121-5.

25. Bell D, Peeling RW (2006): Evaluation of rapid diagnostic tests: malaria. *Nat Rev Microbiol* 4: 34-8.

26. Speers DJ, Ryan S, Harnett G. and Chidlow G (2003): Diagnosis of malaria aided by polymerase chain reaction in two cases with low-level parasitaemia. *Internal Med. J.* 33 (12): 613-15.

27. Scopel KKG, Fontes CJF, Nunes AC, et al. (2004): Low sensitivity of nested PCR using *Plasmodium* DNA extracted from stained thick blood smears: an epidemiological retrospective study among subjects with low parasitaemia in an endemic area of the Brazilian Amazon region. *Malaria J* 3: 8-12.

28. Singh B, Cox-Singh J, Miller AO, Abdullah MS, Snounou G, Rahman HA (1996): Detection of malaria in Malaysia by nested polymerase chain

reaction amplification of dried blood spots on filter papers. *Trans. Roy. Soc. Trop. Med. Hyg.* 90: 519–21.

29. Parajuli K, Hanchana S, Imwong M, Pukrittayakayamee S, and Ghimire P (2009): Comparative evaluation of microscopy and Polymerase chain reaction (PCR) for the diagnosis in suspected malaria patients in Nepal. *Nepal Med. Coll. J.* 11 (1): 23-27.

30. Humar A, Ohrt C, Harrington MA, Pillai D, Kain KC (1997): Parasight F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travellers. *Am. J. Trop. Med. Hyg* 56: 44-48.

31. Hanscheid T, Valadas E (2002): Poor accuracy of rapid diagnostic tests and misdiagnosis of imported malaria: are PCR-based reference laboratories the answer? *J. Clin. Microbiol* 40: 736-737.

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GROUP B STREPTOCOCCUS CARRIAGE DURING LATE PREGNANCY IN ILE-IFE, NIGERIA

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ABSTRACT

This study determined the prevalence of Group B Streptococcus (GBS) in late pregnancy and the antimicrobial susceptibility of isolated GBS as well as the impact of GBS infections on pregnancy related clinical outcome with a view of providing an epidemiological baseline data for policy formulation in the teaching hospital. It is an observational and cross-sectional hospital based study. One hundred and fifty pregnant women from 35-40 weeks of gestation were purposively selected and included in the study from May to December 2010. Vaginal swab samples were aseptically collected from the subjects after informed consent. The samples were assayed for presence of GBS. The susceptibility pattern of the isolated GBS to different antibiotics were assessed using disc diffusion and agar dilution techniques based on the Clinical and Laboratory Standards institute(CLSI) standards. The result showed prevalence of 11.3% GBS vaginal colonization which increased with age. There was no significant association between GBS colonization status and age (p >0.05)), gestational age (p >0.05)), gravidity (p >0.05) and obstetric risk factors (p >0.05)). There was no incidence of GBS infection observed. Although, all (17) the GBS isolates were 100% resistance to penicillin, ampicillin, cefoxitin and clindamycin. Resistance to cefotaxime (11.8%), erythromycin (64.7%) and vancomycin 70.6% were observed. Group B Streptococcus colonization in vagina in late pregnancy has been established in the antenatal clinic of the teaching hospital with the attendant risk to the fetus in the population of those affected. There were high and multiple resistance patterns of the GBS isolates to different antibiotics in this study. This calls for a review of the present hospital policy to include the routine screening of GBS during antenatal visits and surveillance.

Keywords: *GBS, Carriage, Pregnancy and antibiotics resistance.*

INTRODUCTION:

Maternal infections of Group B Streptococcus (GBS) constitute one of the leading pathogens associated with both early and late-onset neonatal sepsis (1). The bacteria are normally found in the vagina and/or lower intestine of 15% to 40% of all healthy.adult women (2). Early onset neonatal sepsis is normally related to vaginal carriage in the motherand subsequent colonization during birth in approximately 70-75% of infants (3). Intrapartum prophylaxis has been established to lead to a 70% decline in the incidence of GBS disease, however, early-onset GBS disease (in infants <7 days old) remains a leading cause of illness and death among newborns (3, 4).

Most data on GBS epidemiology are from Europe and North America and a few cases of GBS infections (5-7) and carriage as a public health problem have been reported from Nigeria (5, 8, 9, 10, 11) and other African countries (12, 13, 14).

This study was designed to determine the prevalence of GBS in pregnancy and the incidence of GBS infections in intrapartum in a tertiary hospital in Nigeria. It also determined the antimicrobial susceptibility of isolated GBS and assessed the impact of GBS infections on the pregnancy related clinical outcome with a view of providing an epidemiological baseline data for policy formulation in the teaching hospital.

MATERIALS AND METHODS

Study Area: The study was carried out at the Antenatal Clinic of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) and the Department of Medical Microbiology and Parasitology, Obafemi Awolowo University (OAU), Ile-Ife Osun, Nigeria from May to December 2010. It was a cross-sectional observational study involving pregnant women between 35 and 40 weeks who attended the antenatal clinic. Pregnant women who

had had antibiotic treatment within the last two weeks prior to recruitment were excluded from the study. The Ethical Committee of the hospital approved the study.

Study Population

One hundred and fifty pregnant women were sampled following the ethical approval of the study. The patients' consents were sought and gained by explaining to them the objectives of the study and the benefits there in. Profomas questionnaires were filled for the volunteers to acquire demographic and other relevant obstetric history.

Specimen Collection

Two swab samples were taken per participant and using a speculum from the vaginal introitus under aseptic condition with a commercially available collection and transport system for aerobes and anaerobes (Charcoal Swabs Amies Plastic Applicator with Rayon Tipped Black Cap, Stone, Staffs, UK). All swab specimens were transported to the Medical Microbiology Laboratory of OAU.

Specimen Culture Isolation and Identification, Microscopy and Antigen Detection

The swab samples were inoculated into selective enrichment broth medium (Todd-Hewitt broth supplemented with 10µ/ml colistin and 15µ/ml nalidixic acid, Oxoid England) and were incubated aerobically at 37^{O}C for 24 hours. After 24 hours incubation, broth cultures were observed for growth (Turbidity) and then sub cultured onto 5% Sheep Blood Agar (Oxoid England) and incubated overnight as above. Suspected GBS isolates were identified appropriately as earlier described (9). GBS antigen was confirmed definitively by serological grouping using Streptococcal group B reagent kit (Oxoid England) testing of selective broth (15).

The results were analyzed using the SPSS software (version 11.0) and evaluated statistically by Chi square, Pearson Yate's correction test. Level of significance was taken to be P - values <0.05. The sensitivity, specificity, positive and negative predictive values and efficiency of the antigen detection test were evaluated against the culture isolation. A pregnant woman was deemed to have been positive for GBS colonization when either or both culture or antigen detection is positive.

Antibiotic Susceptibility Testing

A small number of colonies obtained from each 24 hours old 5% Sheep blood agar plate culture was incubated in Todd-Hewitt broth for 2 hours at 37^{O}C to obtain a McFarland level of turbidity of 10^5 and logarithmic-growth phase culture. These were tested against different antibiotic discs which included penicillin, ampicillin, cefotaxime, clindamycin and

erythromycin and the Minimum inhibitory concentrations were determined based on recommended standards (16, 17) and Streptococcus *pneumoniae* ATCC 6305 was used as control organism and free drug plate was included as negative control.

RESULTS

The study participants reflected the composition of the low risk antenatal group at our hospital in their age, parity, gravidity and gestational age. One hundred and fifty (150) pregnant women participated in the study over a period of six months (May to December 2010). Their age range was 18-42 years with a mean of 30.25 (±4.65) and the age group in years was categorized as younger (18-29) and older (≥30) pregnant women, which were 64 (42.7%) and 86 (57.3%) respectively. Table 1 shows parity and gestational age among other parameters as indicated.

In this study, GBS colonization was confirmed by antigen detection from enrichment broth culture and direct culture isolation of the vaginal swab samples (Table 2). Seventeen (17) out of the 20 pregnant women that were positive for antigen detection by latex agglutination test after 18 hours incubation in enrichment broth were also culture positive. In the remaining 3 antigen positive women, no GBS could be isolated in spite of prolonged incubation (>72hours).

Among the subjects that were colonized with GBS, GBS colonization was higher among older women with age ≥30 years (12.79%) against 9.38% in younger (18-29) women (Table 3). The difference was not statistically significant (X^2= 0.426; P= 0.514). The age distribution of the GBS colonized and non GBS colonized population is shown in Figure 1. The GBS colonization increases as the age increases and a higher colonization was observed in the age range 34-37 years. The age range of GBS positive women was 26-36 years with mean of 30.31(±4.346) while GBS negative women had age ranging from 18-42 years with an average age of 30.17 (±4.741).

Out of the 17 GBS positive pregnant women, 10 pregnant women had their gestational age to be ≤37 weeks but ≥35 weeks while 7 women had their gestational age >37 weeks but ≤40 weeks, the difference was not statistically significant (X^2 = 1.297; P = 0.225)(Table 4). The prevalence of GBS colonization was observed to be higher at 35 weeks gestational age. Among the GBS positive cases, the

prevalence of GBS colonization was higher among the mtltigravida (64.71%) than the primigravida (35.29%) (Table 5). GBS vaginal colonization was not statistically associated with gravidity ($X^2 = 0.375$; P = 0.54).

TABLE 1: CHARACTERISTICS OF THE STUDY PARTICIPANTS

Characteristics	Frequency (%)
Age range	18-42
Age mean (±sd)	30.25(±4.65)
Age group in years	
Younger (18-29)	64(42.7)
Older (≥30)	86(57.3)
Parity range	0-6
Mean (±sd)	1.07(±1.221)
Gravidity	
Primigravida	42(28)
Multigravida	108(72)
Gestational age in weeks	
Range	35-40
Mean (±sd)	36.81(±1.526)

The statistical analysis of GBS carriage in relation to obstetric characteristics is shown in Table 6. Out of the one hundred and fifty studied population, only forty-two (38%) women came to deliver in the hospital eventually. Four (4) out of the forty-two (9.52%) were GBS positive women while thirty-eight (90.48%) were negative cases. Among the 4 GBS positive women, 2 women had gestational age at delivery to be <40 weeks whereas, the other 2 delivered at >40 weeks. The duration of the rupture of membrane (ROM) of the 4 positive pregnant women was less than 10 hours and only one had spontaneous vaginal delivery, the other three delivered through emergency cesarean section due to failure to progress to secondary labour. Twenty seven (27) (96.43%) out of the GBS negative subjects delivered through vagina while the remaining 11 (78.57%) subjects had cesarean type of delivery. However, GBS colonization was not statistically associated with gestational age at delivery, duration of rupture of membrane and mode of delivery (P = 1) (Table 6).

TABLE 2: ANTIGEN DETECTION IN CULTURE POSITIVE AND CULTURE NEGATIVE CASES

Test Result	Culture Positive	Culture Negative	Total
Ag Positive	17 (True Positive)	3 (False Positive)	20
Ag Negative	0 (False Negative)	130 (True Negative)	130
Total	17	133	150

Sensitivity = 100%, Specificity = 95.6%, Positive predictive value = 85%, Negative predictive value = 100%

TABLE 3: PREVALENCE OF GBS VAGINAL COLONIZATION IN RELATION TO AGE

Age (Years)	GBS-positive	GBS-negative	Total
Younger (18-29)	6(9.38)	58(90.62)	64(100)
Older (≥30)	11(12.79)	75(87.21)	86(100)

X^2=0.426; P= 0.514

Out of the 42 subjects that delivered in the hospital, 2 subjects had intrapartum fever; one was GBS positive while the second was GBS negative. Four subjects had vaginal related infection (Vaginitis) out of which one was GBS positive. None of the 4 GBS cases that delivered in the hospital had chorioamnionitis, on the other hand, only one subject out of the GBS negative cases had the infection. One GBS positive subject had fetal distress for an unrelated reason whereas five of the GBS negative also had fetal distress. One subject each from GBS positive and GBS negative gave birth to a neonate with low birth weight. Babies born to 3 GBS positive subjects had normal .Activity, Pulse, Grimace, Appearance and Respiration (APGAR) score. While the baby born to the fourth GBS positive subject had a critically low APGAR score. On the other hand, 35 GBS negative subjects had babies with normal APGAR score while the remaining 3 subjects delivered babies with low APGAR. For the total population that delivered in the hospital, none of the following factors that might contribute to colonization were found to be significantly associated with GBS colonization status (P>0.05) (Table 7).

Antibiotic resistant pattern of GBS isolates by Disc diffusion and agar dilution techniques

Antimicrobial susceptibility pattern of the 17 GBS isolates is shown in Figure 2. All the GBS isolates showed uniform resistance to penicillin, ampicillin, clindamycin and cefoxitin. However, 82.4% of the isolates were sensitive to cefotaxime while 17.6% were resistant. For erythromycin, 35.3% of the GBS isolates displayed both intermediate and total resistance whereas, 29.4% of the isolates were sensitive to the antibiotic. 70.6% were sensitive to vancomycin. Two (11.76%) strains exhibited a multiple antibiotic resistance pattern.

Minimum inhibitory concentrations (MICs) were determined by testing all the 17 GBS isolates against penicillin, ampicillin, cefotaxime, erythromycin and clindamycin by agar dilution method (Figure 3). All the isolates tested had uniform resistance (100%) to penicillin, ampicillin and clindamycin with MIC ≥1milligram per litre (mg/l). Of the 17 GBS isolates tested for cefotaxime susceptibility, 88.2% (15) were susceptible with MICs ≤ 0.5mg/l and 11.8 % (2) were resistant with MICs = 8mg/l. For erythromycin susceptibility, 35.3% (6) were susceptible with MICs ≤ 0.25mg/l and 64.7% (11) GBS isolates had MICs ≥ 1mg/l which were regarded as resistant.

TABLE 4: PREVALENCE OF GBS COLONIZATION IN RELATION TO GESTATIONAL AGE

Gestational age	GBS-positive	GBS-negative	Total
≥35≤37	10(9.43)	96(90.57)	106(100)
>37≤40	7(15.91)	37(84.09)	44(100)

X^2= 1.297; P=0.225

TABLE 5: PREVALENCE OF GBS COLONIZATION IN RELATION TO GRAVIDITY

GBS colonization	Gravidae		Total
	Primi- (%)	Multi- (%)	
Yes	6(35.29)	11(64.71)	17(100)
No	38(28.15)	97(71.85)	135(100)

X^2 = 0.375; P = 0.540

TABLE 6: GBS CARRIAGE RATE AND OBSTETRIC CHARACTERISTICS

Obstetric characteristics	GBS-positive Number (%)	GBS-negative Number (%)	Total Number (%)	P-value
Gestational age at delivery				
<40	**2(9.09)**	**20(90.91)**	**22(100)**	**1**
>40	**2(10.00)**	**18(90.00)**	**20(100)**	
Membrane rupture duration				
<10hours	**4(9.76)**	**4(9.76)**	**41(100)**	**1**
10-18hours	**0(0)**	**1(100)**	**1(100)**	
Type of delivery				
Vaginal	**1(3.57)**	**27(96.43)**	**28(100)**	**1**
Cesarean	**3(21.43)**	**11(78.57)**	**14(100)**	

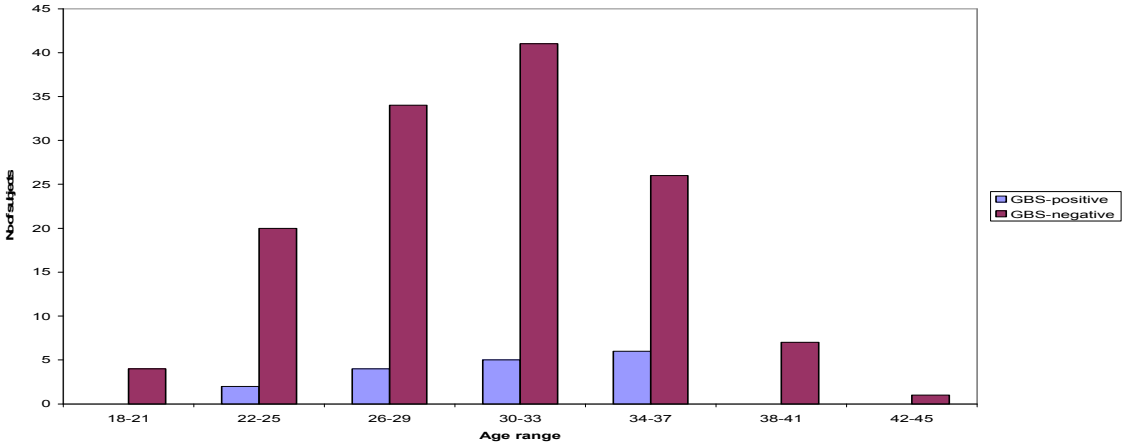

Fig 1: Age distribution and GBS colonization **KEY**: Age range in years

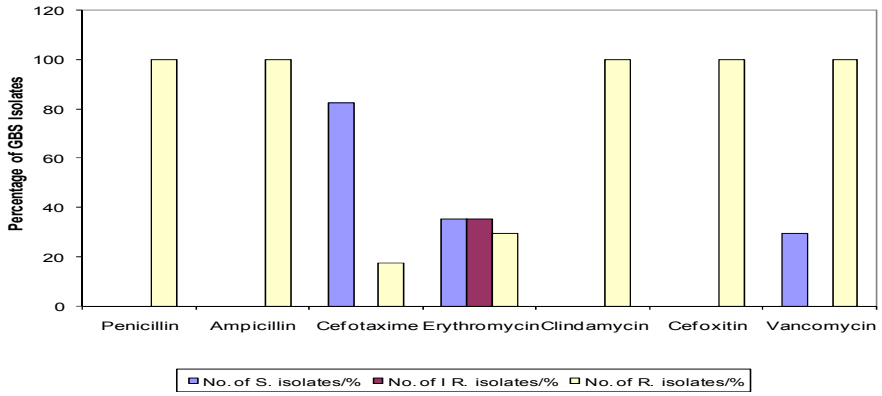

Fig 2: Antibiotic resistant profile of GBS isolates by agar diffusion technique
KEYS: S = Susceptible, IR = Intermediate Resistant, R = Resistant

TABLE 7: GBS CARRIAGE RATE AND REPRODUCTIVE HISTORIES

Reproductive Histories	GBS positive Number (%)	GBS Negative Number (%)	Total Number (%)	P-value
Fever				
Yes	1(50.00)	1(50.00)	2(100)	0.184
No	3(7.50)	37(92.50)	40(100)	
Vaginitis				
Yes	1(25.00)	3(75.00)	4(100)	0.341
No	3(7.89)	35(92.11)	38(100)	
Chorioamnionitis				
Yes	0(0)	1(100)	1(100)	1
No	4(9.76)	37(90.24)	41(100)	
Fetal distress				
Yes	1(16.67)	5(83.33)	6(100)	0.474
No	3(8.33)	33(91.67)	36(100)	
Low birth weight				
Yes	1(50.00)	1(50.00)	2(100)	0.184
No	3(7.50)	37(92.50)	40(100)	
Normal APGAR score				
Yes	3(7.89)	35(92.11)	38(100)	1
No	1(25.00)	3(75.00)	4(100)	

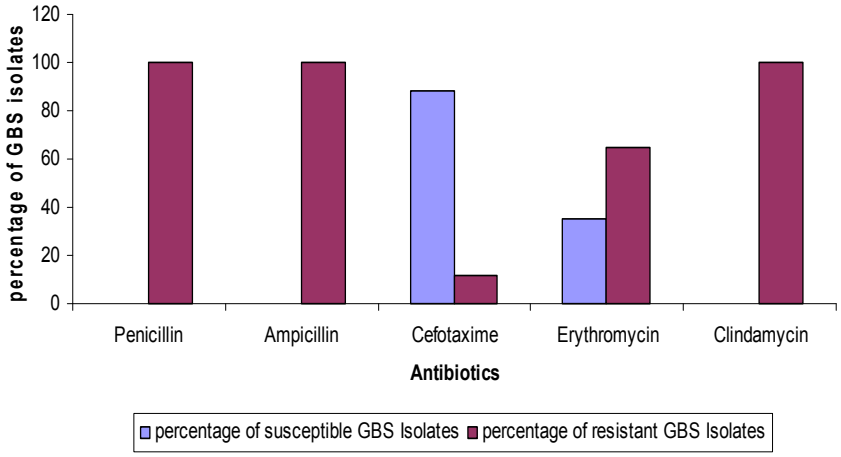

Fig 3: Antibiotic resistance profile of GBS isolates by agar dilution technique

DISCUSSION

This present study has shown the prevalence of GBS (*Streptococcus agalactiae*) colonization in Ile-Ife to be 11.3%. The rapid antigen detection had a sensitivity of 100 percent and a specificity of 95.6 percent in our center which is comparable to 100% sensitivity and 98.37% specificity reported by several other authors (15, 18). The possibility of a higher sensitivity for latex particle agglutination test than the recommended culture isolation cannot be ruled out since agglutination test is independent of the viability of the organism. In this study, there were 3 likely false positive and no false negative cases showing that antigen detection had a sensitivity of 100% and a

specificity of 95.6% with positive predictive value of 85% and negative predictive value of 100%.

In this study, we recorded a higher prevalence of 11.3% GBS vaginal colonization than that reported in Jos (6.6%) (9) and Calabar(9%) (19) but lower than that reported in Ibadan (17.6%) (5) and Zaria (14%) (8). The prevalence rate recorded is comparable to 11% prevalence obtained in Abeokuta (20) and the recent study carried out in Ibadan (10%) (21). Therefore, our study further confirms the presence of GBS colonization of the vagina in late pregnancy in Ile-Ife and corroborated reports from other geopolitical zones of the country establishing carriage status in pregnancy and the consequent risk to maternal and neonatal life. However, there is an urgent need to establish the disease burden arising from this colonization as all studies reported so far failed to address this.

However, in studies carried out in other African countries, higher prevalence rates were obtained, such as: Malawi (16.5%) (12), Gambia (22.0%) (13), Zimbabwe (20-32%), (14) and) Ivory Coast (19.0%) (23). Other countries where a similar rate was reported include; Korea (9.5%) (24), USA (12.2%) (25), Iran (9.1%) (26) and Hong Kong (10.4%) (27). Single vaginal culture and lack of rectal culture can partly explain the low prevalence in our study, although; this may not be a limitation of this study as a higher proportion of GBS have been documented to be isolated from the vagina (12.3%) as compared to the rectum (5%) in Tanzania (28). Higher prevalence rates have been reported in studies that involved rectovaginal sample collections and also women with ruptured membranes (26). The reason for the varying results may be attributed to the fact that GBS maternal colonization varies from place to place. Other factors that may have contributed to this variation include socioeconomic factors, ethnic and genetic factors, variation in clinical practices of samples collected and the techniques used for the sampling. Differences in environmental factors such as hygiene and nutrition may also play a role.

In this study, GBS colonization increases with age. An observation also documented by a study in Ibadan(22) where they reported increase in GBS positivity as age increases. However, this observation was not corroborated by another study reported from Malawi (12) which reported a decrease in GBS colonization as age increases. GBS vaginal colonization rate in this present study does not statistically relate with age nor gestational age ($P > 0.05$). The culture positivity among mothers ≤37 weeks in gestational age was far less than that among mothers >37 weeks which is in agreement with the observation of Raj, *et. al.* (31) that the vaginal colonization of GBS varies during the period of gestation and that screening earlier than six weeks before delivery may not be a true reflection at delivery and may not accurately predict the vaginal colonization at delivery. Other investigators have documented that cultures obtained between one and five weeks before delivery have sensitivity of 87% and specificity of 97% or higher (32).

Although very few GBS isolates were evaluated in this study in a limited population of pregnant women. We are nonetheless alarmed by the high level of resistance observed to penicillin and ampicillin antibiotics which are the first choice of drugs for intrapartum prophylaxis and the challenges which this implies. However, many factors may be responsible for this observation in this environment such as: the ease of procurement of antibiotics in the developing country, the frequent use of antibiotics for therapy and prophylaxis, and other socio-economic factors as documented by Okeke *et al.* (33). The expanded use of beta-lactam antimicrobials in the treatment of several infective clinical syndromes and the easy of purchase over the counter might be the cause of emergence of GBS resistance strains in this environment. A study in Tanzania on GBS in pregnant women documented resistance to clindamycin, erythromycin and penicillin G was found to be 17.6%, 13% and 9.4%, respectively (28). The high rate of erythromycin and clindamycin resistance in GBS in this study is consistent with earlier reported observations (24, 25, and 29) on the increasing resistant trend of GBS to this drugs which underscores the current CDC recommendations that antibiotic susceptibility testing be performed if erythromycin or clindamycin therapy is needed to prevent neonatal GBS infection. GBS erythromycin and clindamycin resistance is as a result of the acquisition of an erythromycin ribosomal methylase (*erm*) gene which encodes a methylase enzyme that modifies the binding site on ribosomal RNA or via the constitutive expression of an *erm*-encoded methylase which results in resistance to erythromycin, clindamycin and streptogramin B drugs ($cMLS_B$ phenotype) (34, 35), we believe that these genes may be responsible for the observations in this study. However, the high susceptibility of 88.2% to cefotaxime, a third generation cephalosporin which was observed in this study, may possibly be due to the limited exposure of subjects to this antibiotics and the relatively expensive nature of the drug which makes it un-attractive for purchase over the counter.

This study demonstrated that detection of GBS vaginal colonization by lance field antigen grouping following enrichment broth culture is more sensitive than direct culture isolation; also it reduces the turnaround time to 18-24 hours for availability of result, unlike the standard culture method that takes about 48-72 hours before result is out.

The high percentage and multiple resistance patterns of GBS to beta-lactam drugs and to clindamycin and erythromycin in this environment need to be further verified through an expanded study with many more women and at several sites with the same study protocol. However, the establishment of carriage status in pregnant women for GBS calls for a review of the present hospital policy on antenatal care to include routine screening and reporting of GBS prevalence as well as monitoring the level of antibiotic resistance in GBS among pregnant women during antenatal visits.

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REFERENCES

1. Apgar BS, Greenberg G, Yen G. Prevention of group B streptococcal disease in the newborn. Am Fam. Physician. 2005: 71(5):903-10.
2. Goodman JR, Berg RL, Gribble RK, Meier PR, Fee SC, Mitchell PD. Longitudinal study of group B streptococcus carriage in pregnancy. Infect Dis Obstet Gynecol. 1997; 5(3):237-43.
3. Boyer KM, Gadzala CA, Burd LI, Fisher DE, Paton JB, Gotoff SP. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. I. Epidemiologic rationale. J Infect Dis 1983: 148:795-801.
4. Benitz WE, Gould JB, Druzin ML. Antimicrobial prevention of early-onset group B streptococcal sepsis: estimates of risk reduction based on a critical literature review. Pediatrics. 2000: 106(2 Pt 1):377-8.
5. Onile, B.A. Group B Streptococcus carriage in Nigeria. *Trans. Rovy. Sco. Trop. Med. & Hyg*. 1980: **74:** 367-370. Onile 1980 (13)
6. Odugbemi, T.O., Ajasin, M.A., and Amodiji, J. Streptococcus group B meningitis. A case report. *East Afr. Med. J.* 1979; **56**: 399-401.
7. Emembolu JO. Endometrial flora of infertile women in Zaria, northern Nigeria. Int J Gynaecol Obstet. 1989 Oct; 30(2):155-9.
8. Uhiara, J.E. Group B Streptococcal carriage among parturient and their neonates in Zaria, Nigeria. Afr. J. Med. 1993 Sci. **22**(3): 79-83.
9. Nsagha, D.S.; Bello, C.S.S. and Kahdakai-Olukemi, V.T. Maternal Carriage in Pregnancy of Group B Streptococcs in Jos: Relation of Endocervical Anorectal Colonization. *Nig. Qt. J. Hosp. Med.* (1997) **7**: 53-56.
10. Olanisebe SB, Adetosoye AI. Determination of asymptomatic carrier rate of beta-haemolytic group B Streptococcus in vaginas of pregnant women in Ibadan, Nigeria. Zentralbl Bakteriol Mikrobiol Hyg A. 1986 Apr; 261(2):248-53.
11. Dawodu AH, Damole IO, Onile BA. Epidemiology of group B streptococcal carriage among pregnant women and their neonates: an African experience. Trop Geogr Med. 1983 Jun; 35(2):145-50.
12. Dzowela, T.; Komolafe, O.O. and Igbigbi, A. Prevalence of Group B Streptococcus colonization in antenatal women at the Queen Elizabeth Central Hospital, Blantyre—A preliminary study. Malawi Medical Journal. 2005. **17** (3): 97-99.
13. Suara RO, Adegbola RA, Baker CJ, Secka O, Mulholland EK, Greenwood BM. Carriage of group B Streptococci in pregnant Gambian mothers and their infants. J Infect Dis. 1994 Nov; 170(5):1316-9.
14. Moyo, S.R.; Modzori, J.; Tswana, S.A.; Mealand, J.A. Prevalence, capsular type distribution, authropometric and obstetric factors of group B streptococcus (Streptococcus agalactiae) colonization in pregnancy. Central Africa Journal of Medicine. 2000: 40(5): 115-120.
15. Park CJ, Vandel NM, Ruprai DK, Martin EA, Gates KM, Coker D. Detection of group B streptococcal colonization in pregnant women using direct latex agglutination testing of selective broth. J Clin Microbiol. 2001 Jan; 39(1):408-9.
16. Clinical and Laboratory Standards Institute. Performance standards for Antimicrobial susceptibility testing; eighteenth informational supplement. 2008. Approved standard M2-A9 and M7-A7. M100-S18 **28**, (1). Wayne, PA 19087, USA.
17. European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Diseases. Determination of

minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clinical Microbiology and Infection, 2000. 6(9): 509-515.
18. Das, A.; Ray, P.; Sharm, M.and Gopalan, S. Rapid diagnosis of vaginal carriage of group B beta haemolytic streptococcus by enrichment cum antigen detection test. Indian J. Med. Res. 2003. 117:247-252.
19. Shabayek SA, Abdalla SM, Abouzeid AM. Vaginal carriage and antibiotic susceptibility profile of group B Streptococcus during late pregnancy in Ismailia, Egypt. J Infect Public Health. 2009;2(2):86-90.
20. Nwachukwu, N.C.; Utsalo, S. J.; Kanu, I. and Anyanwu, E.C. Genital Colonization of Group B Streptococcus at Term Pregnancy in Calabar, Nigeria. The Internet Journal of Pediatrics and Neonatology.2007. ISSN: 1528-8374.
21. Hoppe JE, Lindenau C, Höfler W. Rapid detection of group B streptococci in vaginal swabs of parturients by latex particle agglutination. Zentralbl Bakteriol Mikrobiol Hyg A. 1989 Jan; 270(3):379-84.
22. Donbraye-Emmanuel, O.O.B.; Okonko, I.D.; Donbraye, E.; Fadeyi, A.; Abubakar, M.J, Adebiyi, O.E. and Fashina, N.A. Isolation and characterization of Group B Steptococci and other pathogens among pregnant women in Ibadan Southwest Nigeria. Journal of Applied Biosciences. 2010: 5902. **29**: 1781-1792.
23. Stoll, B.J. and Schuchat, A. Maternal carriage of group B streptococci in developing countries. Pediatrics Infectious Disease Journal. 1998. 17: 499-503.
24. Uh Y, Jang IH, Hwang GY, Yoon KJ, Song W. Emerging erythromycin resistance among group B streptococci in Korea. Eur J Clin Microbiol Infect Dis. 2001 Jan;20(1):52-4.
25. Chohan, L.; Hollier, L.M.; Bishop, K. and Kilpatrick, C.C. Patterns of antibiotic resistance among group B streptococcus isolates: 2001-2004. Infect. Dis. Obstet. Gynecol. 2006: 57492.
26. Mansouri, S.; Ghasami, E. and Shahabi, N. Vaginal Colonization of Group B Streptococci during Late Pregnancy in Southeast of Iran: Incidence, Serotype Distribution and Susceptibility to Antibiotics. J. Med. Sci. 2008. 8(6): 574-578.
27. Tsui, N.H.; Ip, N.; Ng, P.L.; Sohato, D.S.; Leung, T.N. and Lau, T.K. Streptococcal maternal colonization in Hong Kong. Hong Kong Med. J.2009. 15(6): 414-9.
28. Joachim A, Matee MI, Massawe FA, Lyamuya EF. Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. BMC Public Health. 2009 Dec 1; 9:437.
29. Heelan, J.S.; Hasenbein, M.E. and McAdam, A.J. Resistance of Group B Streptococcus to Selected Antibiotics, including Erythromycin and Clindamycin. Journal of Clinical Microb.2004. 42(2): 1263-1264.
30. Itakura, A.; Kuranchi, O.; Morikawa, S.; Matsuzawa, K.; Mizutani, S. and Tomoda, Y. A prospective study on the relationship between intrapartum maternal group B streptococcal concentration and signs of infection in neonates. J. Obstet. Gynecol. Res. 1996. 22: 101-5.
31. Raj, M.; Razali, N. and Sulaiman, S. Screening of Antenatal Mothers and Prevention of Perinatal Group B Streptococcal Infection. JUMMEC 2009. 12(1): 27-30.
32. Yancey, M.K.; Schuchat, A.; Brown, L.K.; Ventura, V.L. and Markenson, G.R. The accuracy of late antenatal screening cultures in predicting genital Group B streptococcal colonization at delivery. Obstet. Gynecol. 1996. 88: 811-815.
33. Okeke, I. N. . Lamikanra, A and Edelman, R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg Infect Dis. 1999 Jan-Feb; 5(1): 18–27.
34. Leclercq, R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. 2002.34: 482-492.
35. Raney, P.M.; Tenover, F.C.; Carey, R.B.; McGowan Jr., J.E. and Patel, J.B. Investigation of inducible clindamycin and telithromycin resistance in isolates of β-hemolytic streptococci. Diagnostic Microbiology and Infectious Disease. 2006. 55:213-218

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EPIDEMIOLOGICAL SIGNIFICANCE OF THE COLONIZATION OF STREPTOCOCCUS AGALACTIAE IN THE ANORECTUM AND ENDOCERVIX OF NON-PARTURIENTS IN JOS, NIGERIA

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ABSTRACT

Knowledge of Group B Streptococcus (GBS) carriage and infections in Africa is very scanty but few cases have been reported in Nigeria in particular. *Streptococcus agalactiae* has been reported to cause infections and diseases in non-parturients and adults ranging from bacteremia, osteomylitis, arthritis, and endocarditis to breast abscess among others, hence the necessity for this study. Fifty six non-pregnant women of different age groups and social status were screened for GBS in Plateau State Specialist Hospital using the Christie, Atkins and Munch-Petersen (CAMP) and hippurate hydrolysis tests. Two (3.6 %) of the 56 women were positive for GBS. The 2 isolates were all from the anorectum. The endocervix yielded no culture. The antibiogram showed that ampicillin is the drug of choice with all isolates (100%) sensitive to the drug. No statistically significant relationship was observed between the clinical and epidemiological characteristics of the patients and GBS carriage (P>0.05). This survey shows a much lower carriage proportion than that reported in Ibadan, Nigeria from non-parturients.

Key words: *Streptococcus agalactiae*, epidemiology, anorectum, endocervix, non-parturients

INTRODUCTION

Streptococcus agalactiae (Lancefield Group B) originally known to cause bovine mastitis (1) is now a recognized pathogen in the neonatal period and an important cause of puerperal sepsis (2, 3, 4). Only recently, however, has the importance of this organism as a cause of infections in adults been recognized (5, 6). In Europe, USA and Australia, this organism has been causing serious havoc to the dairy industry and is occasionally a health hazard especially in the neonatal period and in adults (6, 7, 8, 9). Few cases of GBS infections and carriage have been reported in Nigeria (2, 8, 10, 11, 12, 14) in particular. The intestinal tract appears to be the primary reservoir and likely source of vaginal colonization in pregnant women (10, 11, 14, 15). This relationship makes the genital and lower intestinal tracts known habitats of the organism.

The importance of early detection of asymptomatic people in the community through screening in order to reduce morbidity and mortality from communicable diseases is a vital part of public health (11). We report here the investigation of the epidemiological significance of colonization of Lancefield Group B streptococcus of the anorectum and endocervix among non-parturients in a health institution in Nigeria.

MATERIALS AND METHODS

Fifty six out-patient-non-pregnant women attending the Plateau State Specialist Hospital, Jos, Nigeria, for various health reasons were screened for GBS carriage. The subjects' consent was sought and gained and volunteers were asked to fill a questionnaire which contained information on age, religion, marital status, history of vaginal discharge, recent antibiotic use, miscarriage, family planning, educational and occupational status. Volunteers who could not read or write nor understand English were communicated to, through an interpreter in Hausa (Hausa is the local common language of the study area). The ethical clearance for the study was obtained from the Jos University Teaching Hospital.

Fifty-six anorectal and 56 endocervical swabs (112 swabs in all) were collected through a sterile speculum from each patient using sterile swab sticks and touch light by nurses. Specimens were immediately transferred to the Medical Microbiology Laboratory of the Jos University Teaching

Hospital for laboratory investigation. Cultures were made on 5% Neomycin sheep blood agar plates incubated at 37^{o}C in a candle jar overnight and subsequently sub-cultured to new blood agar plates and incubated as stated above. No transport medium was used since the two hospitals are not far apart. The investigation was carried out using standard microbiological techniques (microscopy, culture, colonial morphology, Gram staining and catalase tests). The isolates were presumptively identified using the CAMP (16) and hippurate hydrolysis (17) tests.

GBS cultures were streaked on blood agar plates and the disk-diffusion method used to carry out the antibiogram with multiple commercial disks (Antee Diagnostic, UK). The plates were incubated overnight at 37^{o}C in a candle jar after which the plates were read for zones of inhibition.

It was assumed that anorectal colonization would be greater than that of the endocervix and that GBS carriage was not going to vary with the clinical and epidemiological characteristics of the patients. Statistical analysis was carried out using SPSS (Statistical Package for Social Sciences) with the chi-square and Fisher exact tests at 95% confidence.

RESULTS

Out of 56 non-pregnant women, 2(3.6%) were positive for GBS. A colonization proportion was discovered per site as follows: endocervix [0(0.0%)] and anorectum [3(3.6%)] (Table 1). One of the GBS isolates was identified using the CAMP test and the other with the hippurate hydrolysis reaction. The characteristic of the subjects were divided into two groups: the epidemiological and clinical characteristics. Table 2 shows the epidemiological characteristics and GBS

carriage, and Table 3 shows the clinical characteristic and the colonization of GBS. There was a positive correlation between the demographic characteristics but none was statistically significant (P= 0.06). Table 4 shows the results of the antibiogram of the common drugs used in Jos for the two isolates. The number of sensitive and resistant *Streptococcus agalactiae* was statistically significant (P<0.05). It was not possible to investigate whether any of the patients was down with any streptococcal group B diseases. It was not also possible to send the GBS isolates for typing in a reference laboratory because of insufficient laboratory preservation facilities.

TABLE 1: GBS CULTURE STATUS ACCORDING TO SITE AMONG NON-PREGNANT WOMEN IN JOS

Site	No of Swabs Collected	GBS Positive No (%)	GBS Negative No (%)	P-Value
Endocervix	56	0(0.0)	56(100)	P>05
Anorectum	56	2(3.6)	54(96.4)	

TABLE 2: GBS CARRIAGE PROPORTIONS AND THE EPIDEMIOLOGICAL CHARACTERISTICS IN NON-PREGNANT WOMEN (n=56)

Epidemiological characteristics	No. tested	GBS positive No (%)
Education		
Literate	50	2(100)
Illiterate	06	0(00)
Occupation		
Employed	51	1(50)
Unemployed	05	1(50)
Religion		
Christian	30	2(100)
Muslim	26	0(00)
Others	00	0(00)
Marital status		
Monogamy	28	1(50)
Polygamy	05	0(00)
Single	33	1(50)
Others (separated, divorced, widowed)	00	0(00)

TABLE 3: GBS CARRIAGE AND THE CLINICAL CHARACTERISTICS IN NON-PREGNANT WOMEN (n=56)

Clinical Characteristics	No. tested	GBS positive No (%)
Age range (years)		
< 19	08	0(000)
20-25	12	0(000)
26-31	27	2(100)
32-36	06	0(000)
> 37	03	0(000)
Vaginal discharge		
Yes	11	0(000)
No	45	2(100)
Recent antibiotic treatment (weeks)		
None	07	0(00)
1	25	1(50)
2	17	1(50)
> 2	07	0(00)
Miscarriage		
Yes	11	0(00)
No	45	2(100)
Family planning		
Practised	21	0(00)
Not practised	35	2(100)

TABLE 4: SENSITIVITY PATTERN OF GBS ISOLATED IN THE JOS ENVIRONMENT

Antibiotic (Conc. mcg)	Sensitive No (%)	Resistant No (%)
Ampicillin (10)	2 (100)	0 (00)
Erythromycin (2)	1(50)	1(50)
Chloramphenicol (10)	1(50)	0 (00)
Tetracycline (10)	0(0.00)	2 (100)
Penicillin (1 iu)	0(0.00)	2 (100)
Cloxacillin (5)	0(0.00)	2(100)
Streptomycin (10)	0(0.00)	2(100)

DISCUSSION

The testing of asymptomatic people in society can help to control communicable diseases morbidity and mortality. Knowledge of GBS infections and diseases in Africa is scanty because of sparsely equipped bacteriology laboratories which results in the organism being missed in the course of diagnosis. Most GBS cases isolated in Nigeria were from pregnant women (8, 10, 11, 13, 14) and infants (8, 18). The isolation of *Streptococcus agalactiae* in the anorectum of non-parturients in this environment should instill awareness in the minds of health officials to review their health control policies towards this organism.

Streptococcus agalactiae was isolated from women attending venereology clinics (19%) in Ibadan, Nigeria (8). A 3.6% colonization proportion in non-parturients in Jos is much lower compared to that observed by Onile (13) in Ibadan, Nigeria, among non-pregnant women. Many reasons affecting sample collection, treatment, site and methods of identification and perhaps environmental factors as well as variations in maternal colonization from place to place have been postulated (8, 10, 11, 13, 14) to support this difference.

The anorectal colonization of 3.6% and an endocervical colonization of 0.00% in this study agree with a discrepancy of anorectal and endocervical colonization in pregnancy earlier reported in this region (10). It has been postulated that anorectal carriage could be due to migration of GBS from the naso-pharyngeal flora to the anorectum (2, 10, 20, 21) since GBS is a normal flora in the nasopharynx.

The carriage proportion of 3.6% in non-parturients is lower than that reported in parturients in this area (10) and other regions in Nigeria (8, 14). The absence of GBS in the endocervix of non-parturients could be due to the lack of ecological conditions, high acidic milieu and a glycogen rich mucosa that prevail in the pregnant cervix (22).

No relationship was established between GBS carriage and the social status of the subjects even though it was higher for literates and did not vary for employment. GBS carriage did not vary according to religion or marital status. Marital status was introduced because polygamy is very common in this area for both Muslims and Christians since the organism is sexually transmitted. The epidemiological character on religion was suggested because it was envisaged that the Muslim cultural habit of washing the 'dirty parts' (genital and peri-anal regions) before ablution (i.e. "tsarki" in Hausa) could have an effect on GBS carriage. The findings of the epidemiological characteristics and GBS carriage were not statistically significant ($P > 0.05$).

In accordance with an earlier report in this area on pregnant women (8, 9), these isolates were concentrated in the age-group 26-31. This could be due to some underlying disease or deficiency (nutritional, hormonal, metabolic or immunological) (7, 23). No relationship was established between vaginal discharge and miscarriage in our study ($P > 0.05$). The two isolates were all from women who did not practice any form of family planning. The effect of contraceptives altering the normal female genital flora has been implicated in increased GBS infections (4). Contraceptives can disrupt the normal vaginal flora, hence the 0.00% observed in women who had practiced any form of family planning (condoms, oral contraceptives, intrauterine contraceptive devices and injections).

Patients who had been on antibiotics for one or two weeks previously still harboured GBS. This could be explained by the fact that these may have been resistant strains. All the two GBS isolates in the Jos environment were sensitive to ampicillin. Ampicillin is safe and can be used for the prophylactic control of GBS infections and diseases in Nigeria. Resistant GBS was statistically significant with respect to the sensitive ones ($P < 0.05$). The high resistance to other antibiotics may be due to antibiotic abuse as Onile (24) had reported on high antibiotic abuse in Nigeria.

In conclusion, further studies on the carriage and acquisition of group B streptococcus in adults is necessary to obtain more information on the reservoirs and sources of the organism. More work is still required to be done on the association between carrier state and disease and control studies. Ampicillin is the drug of choice for the treatment of GBS diseases in Nigeria.

CONTRIBUTION OF AUTHORS

DSN conceived and designed the study; collected and analyzed the data; prepared the draft and final write up; HLFK, JCNA and ALN participated in drafting the manuscript and substantially revised the manuscript; CSSB designed and supervised the study, and substantially revised the manuscript; YTKO supervised the work and revised the manuscript. All authors read and approved the manuscript.

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REFERENCES

1. Ayers, S.H. and Nudge C.S. The streptococci of the bovine udder. *J Infect Dis* 1922; 31: 40-50.

2. Onile, B.A. Group B streptococcal neonatal and infant infections in Nigeria. A review and update. *East Afric Med J 1984*; 61(8): 591-595.

3. Siegel, J.D and McCracken G.H.J. Sepsis neonatorum. *NEJM 1981;* 304: 642-664.

4. Wilson, H.D and Chenwald, E. Sepsis neonatorum, *Pediat Clin North A 1974*; 21:571-582.

5. Small, C.B., Slater L.N., Slvati E.A., et al. Group B streptococcal Arthritis in adults. *A J Med 1984*; 76(367): 577-578.

6. Weiss, R.L. and Matsen J.M. Group B Streptococcal Breast Abscess. *Archive Path Lab Med 1987*; 111:74-75.

7. Bayer; A.S., Chow A.W., Anthony, B.F. and Gruze, L.B. Serious infections in adults due to group B streptococci-clinical and serotypic characterization. *A J Med 1976*; 61:498-503.

8. Onile, B.A. Group B streptococcal carriage in Nigeria. *Trans R Soc Trop Med Hyg 1980*; 74:367-370.

9. Parker, M.T. Neonatal streptococcal infections. *Postgrad Med J 1977;* 53: 598-608.

10. Nsagha, D.S., Bello, C.S.S., Kandakai-Olukemi, Y.T. Hippurate Hydrolysis and Christie Atkins, Munch - Petersen Tests as epidemiological diagnostic tools for *Streptococcus agalactiae* carriage in pregnancy. *East Afri Med J 2000;* 77(1): 32-34.

11. Nsagha, D.S., Bello, C.S.S., Kandakai-Olukemi, Y.T. Maternal carriage in pregnancy of group B streptococcus in Jos: Relation of endocervical and anorectal colonization. *Nig Q J Hosp Med 1997*; 7(1): 53-56.

13. Onile, B.A. Group B streptococcal infections in Ibadan. MD Thesis 1983, University of Ibadan, Ibadan, Nigeria.

14. Uhiara, J.E. Group B streptococcal carriage among parturients and their neonates in Zaria, Nigerian. *Afri J Med Medical Sci 1998*; 22:79-83.

15. Onile, B.A. Review of group B streptococci and their infections. *Afri J Med Medical Sci 1985*; 14:131-143.

16. Christie, R; Atkins, N.E; Munch-Peterson E. A note on a lytic phenomenon shown by group B streptococci. *Aust J Exp Biology Med Sci 1944;* 22: 197-200.

17. Hwang, M. and Ederer G.M. Hippurate hydrolysis method for presumptive identification of group B streptococci. *J Clin Microb 1975*; 1: 114-115.

18. Odugbemi, T.O., Ajasin, M.A and Awodiji J. Streptococcus group B meningitis: A case report. *East Afri Med J 1979;* 56(8): 399-401.

19. Christensen, K., Ripta, T., Agrup, C. and Christenson, P. Group B streptococci in human urethral and cervical specimens. *Scand J Infect Dis 1976*; 8: 75-76.

20. Christensen, K.K., Christensen, P., Flamhole, L. and Ripta, T. Frequencies of streptococci of groups A, B, C, D and G in urethra and cervical swab specimens from patients with suspected gonococcal infection. *Acta Path Microbiol Scand 1974*; 82: 470-474.

21. Franciosis, A. A., Knostman, J.D., and Zimmermann, R.A. Group B streptococcal neonatal and infant infections. *J Pediat 1973*; 82: 707-718.

22. Reid, T.M.S. Emergence of group B streptococci in obstetrics and perianal infections. *BMJ 1975*; 2: 233-235.

23. Ross, P.W., Easmon, C.S.F., Colman, G., Bagg, T., Cumming, C.G., Hostings, M.J.G. Group B streptococcus: Profile of an organism-occasional review. *J Med Microbiol 1984*; 18(2): 139-166.

24. Onile, B.A. (1992). Abuse of chemotherapeutic agents. *Nig Med Pract* 1992; 23 (56): 90-91.

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THE AETIOLOGY OF ENTERIC FEVER IN ABUJA, NORTH CENTRAL NIGERIA

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ABSTRACT

Enteric fever is caused by ***Salmonella enterica serotype typhi***, ***Salmonella paratyphi*** A, B, C, and ***Salmonella typhimurium*** respectively. Of the 2818 blood cultures reviewed, only 90 (3.2%) had positive cultures for ***Salmonella*** species while the 10,007 faecal samples cultured, 159 (1.6%) were positive for ***Salmonella*** species. Identification of isolates was by usual bacteriological techniques including biochemical and serological methods. Percentage occurrence of ***Salmonella*** species in blood and faecal samples show ***Salmonella enterica serotype typhi*** (75.6% and 59.8%), ***Salmonella paratyphi*** A (4.4% and 9.4%), ***Salmonella paratyphi*** B (17.8% and 19.5%), ***Salmonella paratyphi*** C (2.2% and 6.3%) and ***Salmonella typhimurium*** (0.0% and 5.0%). The susceptibility pattern of all the isolates to the eleven drugs used as listed on table iii is highly revealing. For epidemiological status and proper management of patients, it is necessary that appropriate specimens (blood, bone marrow and faecal cultures) are examined and identification of isolates carried out as well as proper sensitivity testing performed prior to treatment for enteric fever.

Keywords: Enteric fever, Blood, Faecal cultures, ***Salmonella*** species, Percentage occurrence.

INTRODUCTION

Enteric fever is caused by ***Salmonella enterica serotype typhi***, ***Salmonella paratyphi*** A, B, C, and ***Salmonella typhimurium*** respectively. Typhoid fever (infection) is an important global health problem with an estimated 16 million cases and 216,000 - 600,000 or more deaths each year, placing typhoid fever in the range of several priority infectious diseases, including ***Human Papilloma Virus*** (HPV), rotavirus and ***Haemophilus influenza*** Type b (Hib). Very high rates of typhoid incidence were found in several DOMI (Diseases of the Most Impoverished) sites, providing further evidence that typhoid continues to be a serious problem (1). It is endemic worldwide but most cases occur in areas of Africa, Asia, and Latin America where sanitation is poor. Although no longer prevalent in the developed world, the aetiological agents continue to cause enteric fever in many parts of the developing world, especially in Asia and northern regions of Africa (2). In 2004, these agents were still estimated to cause approximately 22 million cases of disease and 200,000 deaths each year, primarily in regions where sanitation is poor and clean water is inaccessible (3).

Salmonella enterica serotype typhi is the aetiological agent of typhoid fever, a multisystem disease with protean manifestations and initial lesions in the bowel. ***Salmonella paratyphi*** A & B cause paratyphoid fever.

Typhoid fever still remains a major public health problem in developing countries even in the twenty first century (4, 5). In Nigeria, as in other developing countries of the world, studies have estimated over 33 million cases and 500,000 deaths due to typhoid fever per year (6). Several factors have been attributed to the failure of public health measures to tame the tide of the continuing rise in the incidence, prevalence, morbidity, and mortality of typhoid fever.

It is customary in our society that any feverish condition is first treated for malaria. If this fails, then treatment for typhoid automatically follows and if the patient at this stage fails to respond, it is only then that laboratory investigations are remembered (7). Salmonellosis is responsible for a variety of clinical syndromes including gastroenteritis, enteric (typhoid) fever and extraintestinal manifestations.

Typhoid fever remains one of the most prevalent acute infectious diseases in the developing world including Nigeria. It continues to exist as an endemic disease due to poor (improper) sanitation and low socio-economic status of the people (7).

The clinical diagnosis of typhoid fever is considered to be unreliable (8). A definite diagnosis is obtained when the aetiologic agent, ***Salmonella enterica serotype typhi*** is isolated from faeces, blood, or bone marrow (9).

The aim of this work is to re-emphasize the need to properly identify aetiological agents for enteric fever in our region prior to treatment of same.

MATERIALS AND METHODS

2818 blood cultures and 10007 faecal samples at the Medical Microbiology Laboratory of National Hospital, Abuja was studied.

Blood Culture

The Oxoid Signal Blood Culture System (produced by Oxoid Limited, Wade Road, Basingstoke, Hamsphine, RG24 8PW, England) was used to culture samples of

blood collected from patients where the condition of bacteriaemia is suspected.

FIGURE I: PICTURE OF OXOID SIGNAL BLOOD CULTURE SYSTEM

Collection and Processing of Blood Samples (Blood Culture)

10 ml of blood collected from patient is inoculated into Oxoid Signal Blood Culture bottle. This is a semi-automated system that recognizes bacterial growth in the blood culture by gas production. The inoculated bottle is placed (incubated) at 36^{o}C (+/-) 1^{o}C for 1 hour before inserting the signal device. It is continuously shaken for 24 hours. Incubate at 36^{o}C (+/-) 1^{o}C for at least 7 days (according to manufacturer's instruction).

A positive Oxoid blood culture bottle is indicated by upward movement of fluid into the signal device while a negative Oxoid blood culture bottle is indicated by absence of fluid in the signal device.

All positive bottles are sub-cultured onto Chocolate agar, 3 Blood agar, and MacConkey agar plates and incubated for 24 hours at 36^{o}C (+/-) 1^{o}C. When applicable, it is re-incubated for a further 18 – 24 hours. The second Blood agar plate is incubated at 10% CO_2 enriched environment while the third Blood agar plate is incubated anaerobically (AnO_2) for 48 hours. In 2000, it was stated that in typhoid, ***Salmonella enterica serotype typhi*** can be detected in the blood of 75 – 90% of patients during the first 10 days of infection and in about 30% of patients during the third week (10).

Bacterial isolates were identified by Gram stain, biochemical reactions (KIA, urease test, citrate utilization test), motility test (11). Implicated bacterial isolates by way of the above identification methods were confirmed by sero-typing using ***Salmonella*** polyvalent O and H antisera and monovalent A, B, C, and D sera. The Vi sera is also available for typing (virulence factor).

Antibiotic susceptibility (sensitivity) test using the disc diffusion technique is then carried out with appropriate drugs on confirmed ***Salmonella*** isolates.

Collection and Processing of Faecal Samples

3 - 10 grams of faeces collected over several days (usually 3 days) are preferred (12). However, we made use of only one sample. Faecal samples were cultured

on Salmonella/Shigella Agar (SSA) or Deoxycholate Citrate Agar (DCA) and Selanite Fluid (SF) and incubated at 37°C for 18 – 24 hours. The Selanite fluid preparation is sub-cultured on SSA or DCA and further incubated at 37°C for 18 – 24 hours.

Salmonella and ***Shigella*** species are non lactose fermenters. All non lactose fermenting colonies (NLFs) isolated are subjected to identification as stated above under the blood culture methodology. Identified and confirmed ***Salmonella*** isolates are then subjected to antibiotic susceptibility test using the disc diffusion technique with appropriate antibiotics discs.

RESULTS

A total of 2818 blood cultures were examined, but only 90 (3.2%) had positive cultures for ***Salmonella*** species. The species identified is as stated below;

TABLE I: OCCURRENCE OF *SALMONELLAE* SPECIES FROM BLOOD:

Species	Number of Isolates	% Occurrence
Salmonella enterica serotype typhi	68	75.6%
Salmonella paratyphi A	4	4.4%
Salmonella paratyphi B	16	17.8%
Salmonella paratyphi C	2	2.2%
Total	90	100.0%

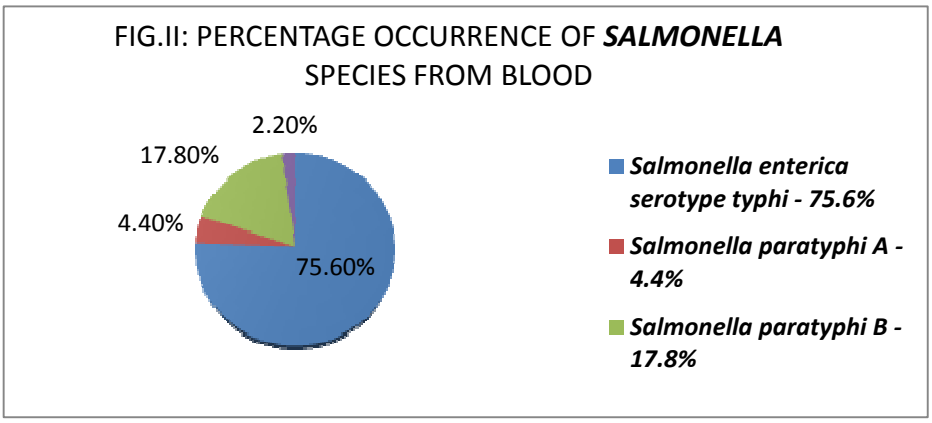

TABLE II: OCCURRENCE OF *SALMONELLAE* SPECIES FROM FAECES

Species	Number of Isolates	% Occurrence
Salmonella enteric serotype typhi	95	59.8%
Salmonella paratyphi A	15	9.4%
Salmonella paratyphi B	31	19.5%
Salmonella paratyphi C	10	6.3%
Salmonella typhimurium	8	5.0%
Total	159	100.0%

Faecal Cultures Result

A total of 10,007 faecal samples were cultured, but only 159 (1.6%) had positive cultures for ***Salmonella*** species. The species identified is as stated below;

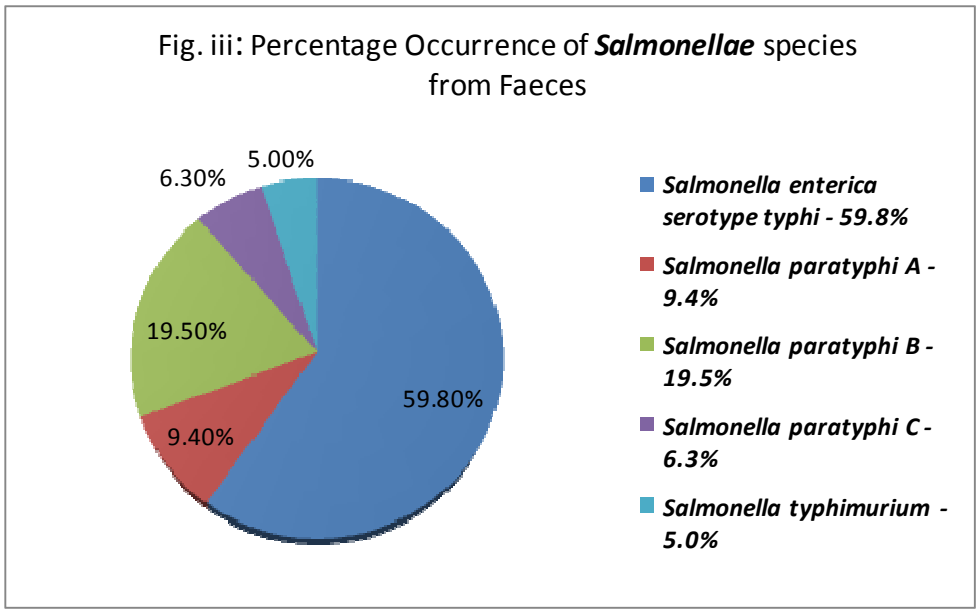

TABLE III: SUSCEPTIBILITY PATTERN OF *SALMONELLAE* SPECIES TO ALL DRUGS

	S. typhi			*S. para* A			*S. para* B			*S. para* C			*S. typhimurium*		
Drugs	**NT**	**NS**	**%S**	**NT**	**NS**	**%S**	**NT**	**NS**	**%S**	**NT**	**NS**	**%S**	**NT**	**NS**	**%S**
Amoxycillin	63	34	54%	9	3	33.3%	20	11	55%	6	2	33.3%	1	1	100%
Ampicillin	30	9	30%	5	2	40%	11	4	36.4%	3	1	33.3%	2	1	50%
Augmentin	129	101	78.3%	10	6	60%	35	18	51.4%	8	6	75%	7	5	71.4%
Cefotaxime	36	34	94.4%	7	7	100%	23	22	95.7%	3	3	100%	4	4	100%
Ceftazidime	50	49	98%	8	8	100%	29	28	96.6%	7	7	100%	8	8	100%
Ceftriaxone	63	60	95.2%	12	11	91.7%	30	30	100%	8	8	100%	3	3	100%
Chloramphenicol	135	81	60%	11	6	54.5%	38	28	73.7%	8	7	87.5%	1	1	100%
Ciprofloxacin	49	48	98%	7	7	100%	20	20	100%	5	5	100%	2	2	100%
Cotrimoxazole	53	36	68%	9	4	44.4%	21	12	57.1%	7	4	57.1%	6	4	66.7%
Gentamicin	63	53	84.1%	7	5	71.4%	32	24	75%	7	4	57.1%	3	3	100%
Tetracycline	51	29	56.9%	10	4	40%	22	10	45.5%	8	5	62.5%	2	2	100%

Note: ***S. typhi* = *Salmonella enterica* serotype *typhi*;** ***S. para* B= *Salmonella paratyphi* B**

***S. para* A= *Salmonella paratyphi* A;** ***S. para* C= *Salmonella paratyphi* C**

S. typhimurium* = *Salmonella typhimurium

NT = Number tested against drug; NS = Number susceptible; % S = Percentage Susceptibility

DISCUSSION

The diagnosis and treatment of enteric fever based on signs and symptoms with a "positive" Widal test will continue to be a subject of controversy (13). In 1986, Edelman & Levine (8) stated that the clinical diagnosis of typhoid fever is considered to be unreliable. Given the diagnostic inadequacies of the Widal test, the use of blood and faecal culture is recommended when suspecting enteric fever (14). Moreover, modern medicine relies on "evidence based medical practice" which is embedded in the actual isolation, identification, and susceptibility patterns of the aetiologic agents from the microbiology laboratory.

In 1984, Hoffman, *et al*., (9) stated categorically that a definitive diagnosis of typhoid fever is obtained when the aetiologic agent ***Salmonella enterica serotype typhi*** is isolated from faeces, blood, or bone marrow.

Of the 2,818 blood cultures in this study, only 90 (3.2%) had positive cultures for ***Salmonella*** species. Of the 90 isolates, 68 (75.6%) were ***Salmonella enterica serotype typhi***, ***Salmonella paratyphi*** A, 4 (4.4%), ***Salmonella paratyphi*** B, 16 (17.8%) and ***Salmonella paratyphi*** C, 2 (2.2%).

Of the 10,007 faecal samples cultured in this study, only 159 (1.6%) had positive cultures for ***Salmonella*** species. Of the 159 isolates, 95 (59.8%) were ***Salmonella enterica serotype typhi, Salmonella paratyphi*** A, 15 (9.4%), ***Salmonella paratyphi*** B, 31 (19.5%), ***Salmonella paratyphi*** C, 10 (6.3%), and ***Salmonella typhimurium***, 8 (5.0%).

In this study, the isolation rate of ***Salmonella enterica serotype typhi*** was higher from blood cultures than from faecal cultures (75.6% from blood and 59.8% from faeces). The reverse was however the case with the other species. Of the ***Salmonella paratyphi*** A isolates, blood cultures had 4.4% as against the 9.4% from faecal cultures. Blood cultures had 17.8% of ***Salmonella paratyphi*** B isolation rate as against the 19.5% from faecal cultures. 2.2% of ***Salmonella paratyphi*** C were recovered from blood cultures as against the 6.3% from faecal cultures. We have no explanations why the above trend was encountered. However, the isolation of ***Salmonella typhimurium*** from faecal samples only was not surprising. In 2007, it was reported that blood cultures are usually negative for ***Salmonella typhimurium***, but faecal cultures remain positive for several weeks after clinical recovery (15).

Conclusion

The laboratory diagnosis or confirmation of enteric fever entails the isolation of the causative (aetiologic) agent and the determination of its sensitivity or resistance patterns to commonly used antimicrobial drugs for proper patient management.

The indiscriminate pretreatment with antibiotics before seeking medical attention in our hospitals and subsequent actual laboratory diagnosis continues to reduce the recovery rate of ***Salmonellae*** from our patient population.

Most times, the clinician may not wait to carry out laboratory test before commencement of treatment. This should be seen and viewed as contrary to modern medical practice which is ''evidence based". Moreover, this impatient attitude by some medical practitioners among other negative effects will certainly increase the rate of bacterial resistance to commonly used antibiotics.

In conclusion therefore, treatment for enteric fever should henceforth be through antibiotics (drugs) dispensed at the Pharmacy based on the Clinician's prescription which rely on proper laboratory diagnostic result clearly stating the aetiology and susceptible drugs

REFERENCES

1. WHO, Report on the Meeting on Typhoid Fever, a Neglected Disease: Towards a Vaccine Introductory Policy, 2007. http://*www.who.int/immunization/sage/typhoid-meeting*.
2. Gordon, M.A., Non-typhoid ***Salmonella*** in sub-Saharan Africa, ***Salmonella***: Biology, Pathogenesis and Prevention. *Am. Soc. for Microbiol, Washington, D.C.* 2009; abstr. S3:2.
3. Crump, J.A., Luby, S.P., Mintz, E.D., The global burden of typhoid fever. *Bull. of W H O,* 2004; ***82***(5): 346 -353.
4. Lin, F.Y., VoAH, Phan V.B., Nguyen, T.T, Bryla, D., Tran, C.T., Ha, B.K., *et al*., , The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta Region of Vietnam. *Am. J Med and Hyg*, 2000; ***62*** : 644 - 648.

5. Otegbayo, J.A., Daramola, O.O., Onyegbutulem, H.C., Balongun, W.F., Oguntoye, O.O., Retrospetive analysis of typhoid fever in a tropical tertiary health facility. *Trop. Gastroenterol*, 2003; ***23***: 9 - 12.

6. Institute of Medicine, New vaccine development: establishing priorities, Washington DC, National Academy Press, 1986.

7. Agbonlahor, D.E., Proper Laboratory Diagnosis and Management of Typhoid fever. A lecture delivered at a seminar on: Modern Techniques in the Clinical Diagnosis and Management of Diseases of Public Health importance in Nigeria at the Nigerian Institute if Medical Research, Yaba, Lagos; 6th August, 2002.

8. Edelman, R., Levine, M.M., Summary of an international workshop on typhoid fever. *Rev. Infec. Dis*, 1986; ***25***: 147-150.

9. Hoffman, S.L., Punjabi, N.H., Rockhill, R.C., Sutomo, A., Rivai, A.R., Pulungsih, S.P., Duodenal String-Capsule Culture Compared with Bone Marrow, Blood and Rectal-Swab Culture for Diagnosing Typhoid and Paratyphoid fever. *J. of Infec. Dis*, 1984; ***149***: 157-161.

10. Cheesbrough, M., Culturing blood: notes on pathogens. In: District Laboratory Practise in Tropical Countries, Cambridge University Press, 2000; Part 2, page 125.

11. Bopp, C.A., Brenner, F.W., Wells, J.G., Strockbine, N.A., ***Echerichia, Shigella*** and ***Salmonella***. In a P.R. Murray., E.J. Baron., M.A. Pfaller., F.C. Tenover & R.H. Yolken (eds). *Manual of Clin. Microbiol*. ASM Press, Washington, DC, 1999; 459-474.

12. Heymann, D.L., Control of Communicable Diseases Manual. 18th edn. Ame. Pub. Health Asso. 2004; 469 – 473.

13. Pang, T., Putchucheary, S.D., Significance and Value of the Widal test in the diagnosis of Typhoid fever in an Endemic Area. *J. Clin. Pathol*, 1983; ***36***:471-475.

14. Gonzalez, A., Malo, Q., Roman, M.I., Molina, J., Perez, M.T., Domingu, B.N., Microbiologic Diagnosis of Typhoid Fever; Report on an Outbreak. *Enfermedades Infeccioses Microbiol. Clin.* 1994; ***12***: 372-377.

15. Jawetz, Melnick and Adelberg, Enterocolitis: In - Medical Microbiology, McGraw-Hill Companies, Inc. International Edition, 24th edition, 2007; Chapter 16, page 259.

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THE INFLUENCE OF GESTATIONAL AGE ON THE LOSS OF MATERNAL MEASLES ANTIBODIES IN NEWBORN INFANTS IN NORTH-EASTERN NIGERIA: A CALL FOR A REVIEW OF MEASLES IMMUNIZATION SCHEDULE

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ABSTRACT

Background: Maternal measles antibodies (MMA) are actively transferred in mother-infant pairs during third trimester of pregnancy. Gestational age (GA) affects the levels of MMA such that longer GA may result in infants starting out with high levels of MMA.

Objective: To determine the influence of GA on the loss of MMA in newborn infants in North-Eastern Nigeria.

Method: A prospective study was conducted on newborn infants at Maiduguri; sera were collected at birth and at six months of age. Enzyme linked immunosorbent assay (ELISA) was used to measure MMA while GA was determined using the last menstrual period, ultrasound scan reports and the Dubowitz criteria.
Results: Seventy eight newborn infants were enrolled. Seventeen (89.5 %) preterm, 43 (95.6 %) term and 14 (100 %) postterm had protective levels of MMA at birth. Two (10.5 %) preterm, nine (20.0%) term and two (14.3 %) postterm had protective MMA at six months of age. Comparison of mean MMA at birth and at six months of age was significant (p = 0.005), however, it was independent of GA of the newborn infants.

Conclusion: Significant decline of mean MMA levels was seen in these infants at six months of age, which was independent of their GA. These infants may be prone to measles at an earlier age (less than six months). Therefore, the current recommendation of measles immunization to infants at nine months of age may require reconsideration.

Keywords: maternal measles antibodies, gestational age, measles immunization, newborn infants.

INTRODUCTION

Measles is the most common vaccine preventable cause of death in the world. The World Health Organization (WHO) estimates that 8.703 million disability adjusted life years (DALY) due to measles and 256,000 measles associated deaths still occur yearly (1). Developing countries are worst hit (1). The mainstay of measles control is measles vaccine. Since 1976, the WHO has recommended that measles vaccine be integrated into routine health services and be administered at nine months of age in developing countries (2). This recommendation was based on studies demonstrating seroconversion rates of over 90% in children nine months of age or older in developing countries (3). In Nigeria, measles vaccine is also being administered at nine months of age. However, 10-15% of measles occur in infants at the age of six-eight months, that is, before they can be immunized against measles (4). Therefore, the question arises whether administration of measles vaccine should be earlier than nine months in order to protect this group of infants. Most infants are protected against measles by MMA but are susceptible to measles when these antibodies fall to unprotective levels (3, 5). As MMA persist in infant, measles vaccine cannot be given because these

antibodies would interfere with its uptake and subsequent seroconversion (5).

Trans-placental MMA transfer occurs mainly in the third trimester of pregnancy and the level of MMA in a neonate is directly proportional to gestational age (6). Study of term infants has demonstrated that MMA are lost to an unprotective levels by seven months of age in developing countries (3, 5). Therefore, measles vaccine administered around that time, theoretically, would be effective in those infants. Keeping in mind that the degree of MMA transfer is related to gestational age, preterm infants are likely to have lower MMA at birth. Thus, preterm infants are likely to become seronegative earlier than term infants. This presumably could be due to early interruption of intrauterine life associated with preterm deliveries. Very few studies have been done to verify this hypothesis (7). If this is so, it stands to reason that, preterm infants would require measles vaccine earlier and also would seroconvert adequately if given measles vaccine at an earlier age like term and postterm infants (8). In view of the above, this study was undertaken to estimate and compare the levels of MMA in preterm, term and postterm infants at birth and six months of age.

SUBJECTS AND METHODS

The study was conducted on 19 preterm, 45 term and 14 postterm infants delivered at the University of Maiduguri Teaching Hospital (UMTH), Borno State. After clearance from the Medical Research and Ethics Committee of UMTH, informed consent from parent was obtained. Gestational age at birth was assessed from the last menstrual period and ultrasound scan reports where available and correlated with the Dubowitz criteria (9). Each infant was allotted a serial number at birth. Three millilitres of cord blood was collected in a sterile bottle and serum separated by centrifugation at 5000 rpm for five minutes. Serum samples collected were stored in a refrigerator at -20°C until the time of MMA assay. Each infant was thereafter followed up periodically at the well baby clinic. At sixth month of postnatal life, another blood sample was collected by venepuncture from each infant aseptically and stored in a similar manner as above.

On completion of collection, the samples were assayed for MMA using ELISA (Demeditec diagnostic Gmbh Kiel Germany) in accordance with the manufacturer's instructions.[10] Optical densities (OD) of reactions in the well plates were read in an automated analyzer at 450 nanometre (nm) wavelength, MMA titres were obtained by plotting graphs of OD against measles IgG concentrations. On the basis of manufacturer's recommendations, protective titres for MMA were defined as the levels of MMA >12 U/ml, and unprotective titres as levels of MMA ≤ 12 U/ml (10).

Data analysis. Appropriate statistical method was used to analyzed the data obtained from this study using SPSS statistical software version 16, Illinois, Chicago USA. A p value < 0.05 was considered significant. Tables were used appropriately for illustrations.

RESULTS

The study group consisted of 78 newborn infants, out of which 40 (51.3 %) were males and 38 (48.7 %) were females. The male to female ratio was 1.05: 1. Of these newborn infants, 19 (24.4 %) were preterm, 45 (57.7 %) term and 14 (17.9 %) posterm infants respectively (Table 1). Table 2 indicates that 17 (89.5 %) preterm, 43 (95.6 %) term and 14 (100 %) postterm newborn infants at birth had protective MMA. Whereas, two (10.5 %) preterm, nine (20.0%) term and two (14.3 %) postterm were having protective MMA at six months of age. Table 3 shows that the overall comparison of mean MMA at birth and at six months of age was significant (p = 0.005). However, the comparison of mean MMA at birth and at six months of age was independent of GA of the infants at birth (p = 0.158) and at six months of age (p = 0.83).

DISCUSSION

Majority of the preterm, term and all postterm newborn infants in this study were found with protective levels of MMA at birth. With Preterm having lower but protective levels and postterm having higher protective MMA than term deliveries. This was the observation made in previously conducted studies that compared the influence of gestational age on MMA in different countries (5-7). This may be related to decreased MMA from haemodilution, and unequalled transfer of measles IgG subclass mostly seen in the first and second trimester of pregnancy (11). Since MMA in foetal circulation increases until the time of birth (5, 11), it seems reasonable to assume that these MMA will be reduced as gestational age decreases. Also, the placentae of infants born before 37 weeks of GA may have fewer mature receptors for MMA than those of full-term infants (5). All these could lead to lower levels of MMA being transported across the placental barrier to the foetus. In view of this, some authors suggested that GA was the single most important factor in transplacental transfer of MMA between mother and child (7).

TABLE 1: GESTATIONAL AGE AND SEX DISTRIBUTION OF THE 78 INFANTS

GA (Weeks)	Male	Female	Total
Preterm (< 37)	12	7	19
Term (≥37 < 42)	20	25	45
Post term (≥ 42)	10	4	14
Total	40	38	78

GA= Gestational age

TABLE 2. DISTRIBUTION OF MATERNAL MEASLES ANTIBODIES ACCORDING TO GESTATIONAL AGE OF THE NEWBORN INFANTS

MMA (U/ml)	Preterm infants (n= 19)		Term infants (n=45)		Postterm infants (n= 14)	
	Birth n (%)	6 months n (%)	Birth n (%)	6 months n (%)	Birth n (%)	6 months n (%)
Protective levels	17 (89.5 %)	2 (10.5 %)	43 (95.6 %)	9 (20.0%)	14 (100 %)	2 (14.3 %)
Unprotective levels	2 (10.5 %)	17 (89.5 %)	2 (4.4 %)	36 (80.0 %)	0 (0 %)	12 (85.7 %)

MMA=Maternal measles antibodies

TABLE 3. COMPARISON OF GESTATIONAL AGE AND MEAN MATERNAL MEASLES ANTIBODIES AT BIRTH AND SIX MONTHS OF AGE

	Mean maternal measles antibodies ± SD (U/ml)		
GA (Weeks)	Birth	Six months	p- value
Preterm (<37)	188.16 ± 87.99	7.21 ± 5.93	0.000
Term (≥37 <42)	207.27 ± 77.44	7.44 ± 8.07	0.000
Post term (≥42)	239.00 ± 29.90	8.79 ± 10.01	0.000
p- value	0.158	0.830	

t = 14.194, p= 0.005 GA= Gestational age

In the present study, no significant difference was noted in the levels of MMA transferred in preterm, term and postterm neonates at birth. This is consistent with the finding of a study conducted in India (7). Therefore; it can be derived that though important, GA may not be the only factor affecting tansplacental MMA transfer in mother-infant pairs. It is postulated that the levels of measles antibody present in a mother at the time of delivery besides maternal age and nutrition, also depends on measles virus exposure and measles vaccine induced immunity (5).

In this study, it was found that at six months of age, 89.5%, 80.0% and 85.7% of preterm, term and postterm infants were having unprotective MMA. This observation corroborated that of other workers (7) where the loss of MMA was linked to its normal catabolism with the passage of time. The present study also found that there is a significant decline of mean levels of MMA from birth to six months of age in preterm, term and posterm infants, which was independent of their GA. This is similar to the observation made in Congo where GA of neonates did not contribute significantly to the loss of MMA in

infancy (12). On the contrary, some authors have shown that MMA in neonates at term onward may persist throughout the first year of life, only to decline to unprotective levels after the first birthday (5). This difference may be explained by the fact that the latter study was undertaken in developed country, while the present study was carried out in a developing country, where the loss of MMA have been postulated to occur at a faster rate (3, 5, 12).

Some of the reasons advanced for the early decay of MMA in developing countries were sickness and repeated blood sampling for diagnostic tests in neonates. This results to the removal of measles antibody containing plasma and replacement by packed red blood cells does not replace the measles antibody lost (5). Additionally, the higher incidence of infections leads to hyper catabolic state causing faster catabolism of MMA in these infants (5). Cases of respiratory tract infections and diarrhoeal diseases were reported in some of the infants in this study. This could have contributed to the rapid decline of MMA in our study population.

Regarding the optimal age of measles immunization of infants in the current study, 89.5%, 80.0% and 85.7% of preterm, term and posterm infants are likely to seroconvert at six months of age. The measles vaccination failure rate thus would be 10.5%, 20.0% and 14.3% for preterm, term and postterms infants respectively. Presently, the vaccination failure rate at nine months in developing countries is about 5-10% (13). For successful uptake of measles vaccine, two conditions should be fulfilled. Firstly, MMA should be absent and/or should not interfere with uptake. Secondly, the immune system of the patient must be capable of mounting an adequate immune response.

The present study revealed that most of the infants lose their MMA by six months of age, with preterm infants having the highest percentage. As far as the second factor is concerned, Pabst *et al* (14) have pointed out that the capacity to produce antibodies to a variety of antigens increases as a function of age. Thus, it is possible that an immature immune system would cause diminished antibody response to measles vaccine in preterm infants. However, a study conducted on the response of preterm infants to Varicella vaccine have demonstrated that by four months of age, the response of preterms was comparable to term and postterm infants (15). This comparable immune response can be explained by the fact that by virtue of their early exposure to extrauterine life, the immune system of a preterm neonate matures rapidly and may be capable of mounting immunological response required for satisfactory uptake of vaccine (8). Therefore, it is quite possible that the measles antibody response to measles vaccine given at six months of age would be adequate.

Conclusion

A significant decline in transplacentally transmitted mean MMA levels was seen in preterm, term and postterm infants over a period of six months after birth, which was independent of their GA. These infants irrespective of their GA are thus prone to measles at an earlier age (less than six months). Therefore, the current recommendation of immunizing infants at nine months of age may require reconsideration.

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REFRENCES

1. Janison, D.T., Crease, A., Prentice, T. The Double Burden: Energing Epidemics and Persistent Problems. World Health Report, Geneva, *World Health Organization*, 1999; p 19.
2. Galazka, A.k., Lauer, B.A., Henderson, R.H., Keja, J. Indications and contraindications for vaccines used in the expanded program on immuniza-tion. *Bull WHO* 1984; 62: 357-66.
3. Anonymous. Measles immunity in the first year of life and the optimum age for vaccination in Kenyan children. Collaborative study by the Ministry of Health of Kenya and the WHO. *Bull WHO* 1977; 55: 21-2.
4. United Nations Children's Fund. The State of the Worlds' Children, 1997. New York, Oxford University Press, 1997; pp 77-103.
5. Victor, M.C., Peter, M.S., Roland, W.S. Factors determining prevalence of maternal antibody to measles virus throughout infancy: A review. *Clin Infect Dis* 2000; 31:110-19.

6. Toivanen, P., Mantvejarvi, R., Mirvonen, T. Maternal antibodies in human fetal sera at different stages of gestation. *Immunology* 1968; 15: 392-95.
7. Rau, A.T., Dhulia, A., Wilson, C.G., Chopra, G.S., Sarkar, P.K. Transaplacentally Transmitted Anti-Measles Antibodies in Term and Preterm Infants. *Indian Pediatrics* 2002; 39:282-88.
8. Dancis, J., Osborn, J.J., Keinz, H.W. Study of the immunology of the newborn infant and antibody formation in the premature infant. *Pediatrics* 1983; 212: 151-52.
9. Dubowitz, L., Dubowitz, V. Gestational Age of the Newborn. Reading MA, Oxford University Press, 1977.
10. Product information instruction manual. Enzyme immunoassay for the detection and the quantitative determination of human IgG antibodies against measles virus in serum and plasma. Demeditec diagnostics 2009. P. 1-8.
11. Kamar, S., Chowdhury, O., Murshed, M., Hasan, R. Effect of Gestational Age and Nutrition on Transplacental Transfer of Measles Antibody. *Med Today* 2010; 22:1-5.
12. Dubois, F., Waldman, R.J., Mann, G.F. Loss of maternal measles antibody during infancy in an African city. *Int J Epidemiol* 1989; 18: 264-66.
13. Job, J.S., John, T.J., Joseph A. Antibody response to measles immunization in India. *Bull WHO* 1984; 62: 736-37.
14. Pabst, H.F., Spady, D.W., Marusyk, R.G., Sprader, T.S. Reduced measles immunity in a well vaccinated population. *Pediatr Inf Dis J* 1992; 2: 524-25.
15. Lipton, S.V., Brunnel, P.A. Management of Varicella exposure in NICU. *JAMA* 1989; 261: 1782-84.

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SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PATIENTS DIAGNOSED WITH HIV IN ACCRA AND KUMASI METROPOLIS

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ABSTRACT

Human Immunodeficiency Virus (HIV) is RNA virus that causes Acquired Immune Deficiency Syndrome (AIDS). In Ghana the AIDS epidemic is spreading very fast in densely populated areas with higher numbers of cases occurring in the southern regions especially the densely populated capitals such as Kumasi, Koforidua and Accra as well as mining towns like Obuasi and Tarkwa, and in border towns.

Data was collected from Accra and Kumasi on socio-economic backgrounds such as age, sex, education, marital status, household size, among others with a structured questionnaire and analysed using statistical Package for Social Sciences (SPSS) version 16.0

About 72% females and 28% males were interviewed with close to 74% lying between 22 to 40 age brackets. Also 63% of these completed JHS/Middle school, Seventeen (36.1%) are married people, 15 (31.9%) widowed. Majority of them are Akans who are also Christians with different denominations. Among these people, 20 (42.6%) of them are unemployed, and 3 (6.4%) claimed to be self-employed in various disciplines. Most of them said they use condoms as contraceptives in order to prevent the spread of the disease.

Income level of the respondents predominantly lies between GH¢50.00- GH¢450.00 per annum.

Some of the interventions for preventing the disease include promoting abstinence and faithfulness, promoting reductions in the number of sexual partners, encouraging delays in the onset of sexual activity among others.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is RNA virus that causes Acquired Immune Deficiency Syndrome (AIDS). The virus destroys the T-helper cell which fights opportunistic infections such as pneumonia and tuberculosis (TB). One can contract the virus for a long time without showing any symptoms of the disease, yet during this period, transmission is possible especially through sexual contact with people. An infected woman can also transmit the disease to her infant during pregnancy, delivery and breastfeeding. HIV can also be spread through blood transfusions or blades that have been in contact with blood of an HIV infected person. AIDS itself is defined in terms of how much deterioration of the immune system has taken place as seen by the presence of opportunistic infections.

Two main transmission mechanisms account for most new HIV infections globally. These are heterosexual contact and mother-to-child (MTC) transmission.

About 80% of infections are transmitted through heterosexual contact (1). Although the probability of transmitting HIV during intercourse can be quite low, a number of factors increase the risk of infection dramatically. One is the presence of a sexually transmitted disease (STD) such as syphilis and gonorrhoea in either partner during unprotected sex.

These diseases form ulcers and sores that facilitate the transfer of the virus.

In Sub-Saharan (SSA) Africa, the toll for the disease on the general population is increasing daily at an exponential rate. It is estimated that about 2 million people were newly infected with HIV in SSA in 2007. This makes the total number of people living with HIV in SSA to hover around 22 million, which is 6% of the global total of 32.9 million, according to (2). The HIV/AIDS epidemics in SSA vary from country to country with most countries in Southern Africa such as Botswana, Lesotho, Namibia, South Africa, Swaziland, Zambia and Zimbabwe having prevalence rate exceeding 15 (2)

Although HIV/AIDS is prevalent in all population groups, data from most countries suggest that it is more pronounced among those who are within the reproductive and productive age group. For example, data from the Untied States of America (USA) show that among youths aged 20 to 24 years, 64% of reported HIV infections occurred among young men and 36% among young women. While among youths aged 13 to 19 years, 57% of reported HIV infections occurred among women and 43% among young men (3).

Giri et al (4) in their study on the socio-demographic characteristics of HIV infection in northern Italia 134 patients testing positive to HIV antibody, revealed that adults male appeared to have the highest HIV rates. A similar study in Kuala Lumpur based on data collected between 1987 and 1995 found that over two third of those infected were males (5).

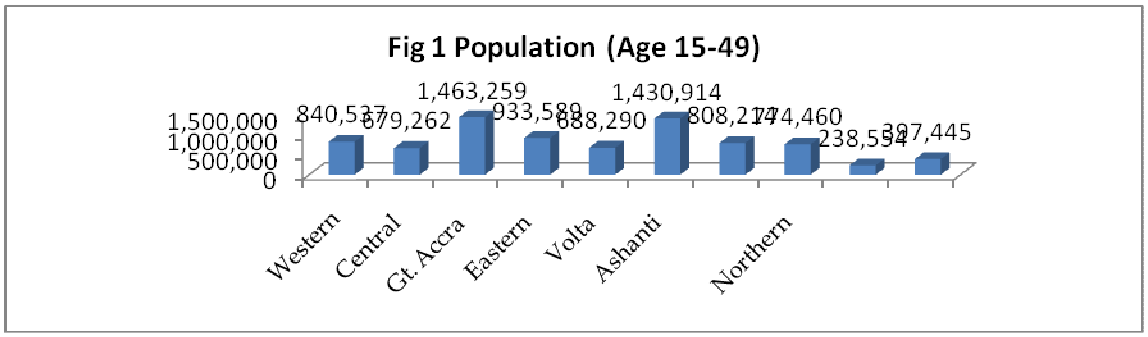
In the last one-decade, the prevalence rates of the HIV/AIDS epidemic have been found to be higher in women than in men (6,7, 8,9 and 10). Since the first clinical evidence of AIDS was reported more than two decades ago, HIV/AIDS has spread to every corner of the world. Still growing, the epidemic is reversing development gains, robbing million lives, widening the gap between rich and poor, and undermining social and economic security

The Economy of Ghana and HIV/AIDS

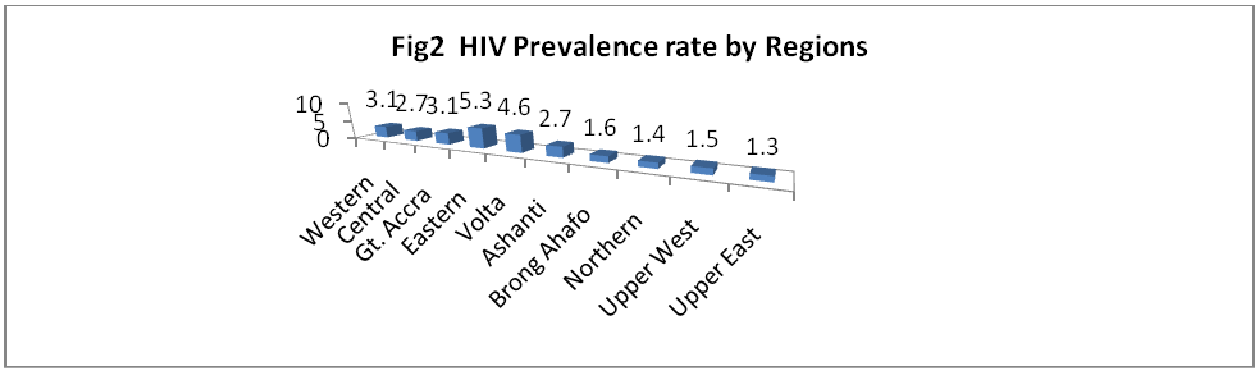
Ghana has a population of about 24 million with growth rate of 2.5% and life expectancy of 57 years. The AIDS epidemic is spreading very fast in densely populated areas. Higher numbers of cases occur in the southern regions especially the densely populated capitals such Kumasi, Koforidua and Accra as well as mining towns like Obuasi and Tarkwa, and in border towns. Poverty level in Ghana by 1999 was estimated to be 29.4% with rural-urban variations. Poverty and other economic pressures on individuals in the cities constitute major factors for the spread of HIV/AIDS. Other factors which contribute to the spread are high youth unemployment, limited job opportunities, rural – urban migration for jobs, market-places, street children, commercial sex workers, itinerary traders, long-distance drivers/truckers as well as Accra – Tema air and sea ports being the gateways for land locked and war-torn countries.

The above among other factors, lead to worsening of poverty situations in rural areas, break-up of traditional family system and norms, promotion of transactional sex and sexual relations and potentiating the rate of contracting and spreading HIV/AIDS in the communities. In every community, sex workers, truckers, migrant/seasonal workers, young girls, orphans and unemployed youths, constitute the major vulnerable groups to contracting and or spreading HIV/AIDS due to their economic and social activities. However, in the 1990s, HIV became the third after TB and Respiratory Track Infections (RTI) as the cause of adult death in developing countries.

Youth Population and HIV/AIDS Prevalence in Ghana



Source: (11).



Source: (11)

The figures above show the general population of Ghana at the youth level and the prevalence rate of HIV/AIDS for ages 15-49 years. It is obvious that majority of these youths are in the Ashanti and Greater-Accra regions of Ghana with more than 1.4 million people living in these regions. Because of this activities and youthful exuberances are higher and therefore more prone to AIDS compared to the others though currently, the prevalence rate from fig 2 depicts a contradictory picture. Because of youthful exuberance, studies to ascertain the socioeconomic backgrounds of the regions showing majority of these youth is laudable since one person can more than double the transmission of the virus. Therefore, the objective of this study is to describe the socioeconomic characteristics of some selected patients living with AIDS in Accra and Kumasi metropolis.

METHODODLOGY

This survey formed part of a longitudinal study on nutrition intervention among adults living with HIV. Data was collected from Accra and Kumasi. Accra is in the Greater Accra Region of Ghana. It is located in the southern part of Ghana along the coast of Atlantic Ocean. It borders Volta to the East, Central to the West and Eastern regions to the North. Two major Health Centres, Achimota and Ridge were consulted for the sample collection in Accra. Kumasi is in the Ashanti region of Ghana and it forms borders with Western, Central, Eastern and Brong-Ahafo region of Ghana. In Kumasi, sample was collected at Young Men Christian Association (YMCA) office near Amakom. Prior to the data collection, a short education was given to these patients on the study.

Information on socio-economic backgrounds such as age, sex, education, marital status, household size, among others was collected from the respondents with structured questionnaire. Data collection started in 2007 and ended in 2009.

Data was analysed using statistical Package for Social Sciences (SPSS) version 16.0 and presented using graphs and descriptive statistics including frequencies, means and percentages.

RESULTS

Age, Sex and Education of Respondents

Out of 47 HIV subjects that fully participated in the project 34 (72.3%) were females, and 13 (27.7%) males. Also 73.9% were between the ages 22 to 40 but most of them were 33 years of age. The average age of the distribution was 35.8 years (35.8±5.8). Among these people, 29 (63%) completed JHS/Middle school, 7 (15.20%) finished SHS, 2 (4.3%) had no formal education, the others completed vocational/secretarial school, primary and non formal schools

Table 1 Socio Demographic Characteristics

Factor	Frequency	percentage
Age (years)		
20-40	**35**	**74.5**
41-60	**12**	**25.5**
Sex		
Male	**13**	**27.7**
Female	**34**	**72.3**
Education		
None	**2**	**4.2**
Primary	**3**	**6.4**
Middle	**29**	**61.7**
SHS	**8**	**17.0**
Vocational	**4**	**8.5**
Non formal	**1**	**2.1**

Seventeen (36.1%) of the subjects are married people, 15 (31.9%) widowed, others are single, cohabiting or divorced.

Ethnic Group and Religious Affiliation

The ethnic groups of the respondents are as follows; Twenty-eight (59.5%) are Akans mainly of Ashante, Akyem and the Akuapim tribes, only a handful of them are of different tribe such as Frafra, Brong, Fanti, Ga, Ewe, Hausa and Dagarti. In terms of religion, 43(91.4%) are Christians of different denominations such as Apostolic Church, Assemblies of God, Baptist, Methodist, Jehovah Witness, Pentecost, Seventh Day Adventist (SDA) and Presbyterian Church while 4 (8.6%) are Moslem.

Table 4: Religious Denomination

Religion	No	%
Christianity	**43**	**91.4**
Moslem	**4**	**8.6**
Total	**47**	**100**

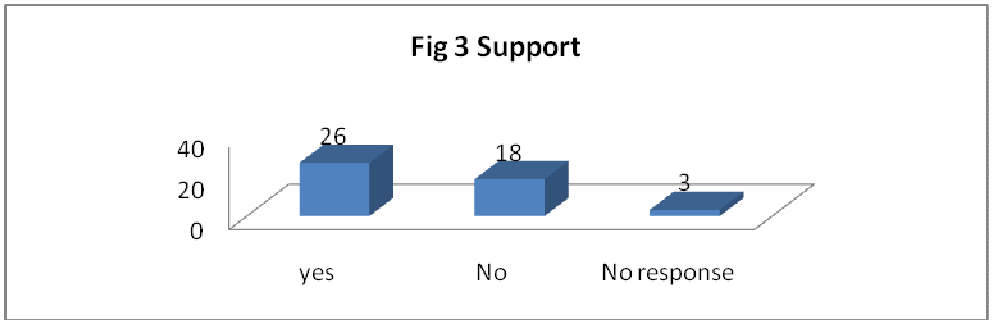
Household size and Occupation

Thirty-one (66%) of the respondents have total household size of 1-5 and the others have more than six people living in the house together. Among these people, 20 (42.6%) of them are unemployed, 15 (31.9%) are traders, 3 (6.4%) claimed to be self-employed in various disciplines mostly artisans and craftmen. Others are in various outfits such as food processing, sewing, welding, hairdressing, and farming with a handful working in the government institutions.

Support from somebody

Twenty six (59.1%) said they receive support from their close relatives and 18 (40.9%) depend solely on their own efforts. As to the nature of support, 21(44.7%) said they receive financial support, others receive support such as food, clothing, medication and shelter from philanthropists and NGOs. See fig 3.

Figure 4 Support from somebody

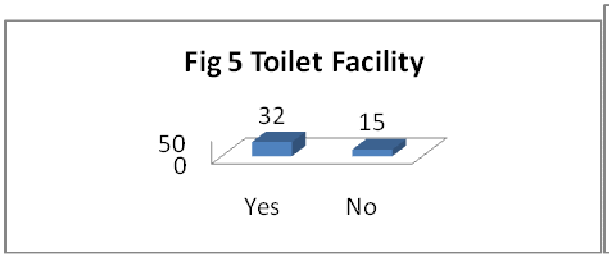


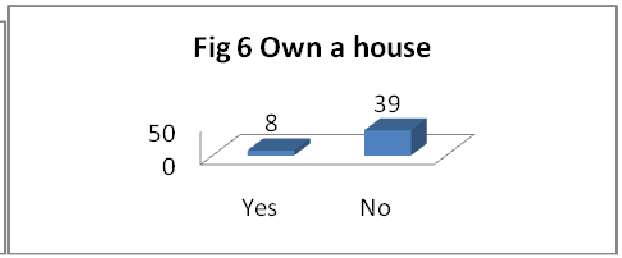
Housing Status

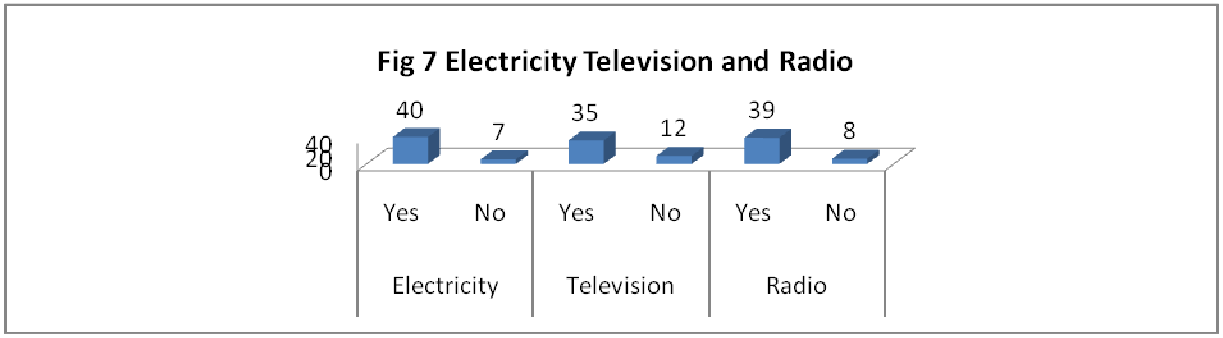
Eight (17%) own houses while 39 (83%) do not own any house. Among those who do not own house, 23(43.9%) rent room and 16(34%) lodge with their friends, others did not disclose their housing status.

Those who rent or lodge with friends said they are connected to the electricity which was disclosed by 40 (85%) of the respondents with only 7 (14%) having no access to electricity. Thirty-two (68.1%) said they have access to potable drinking water (pipe-borne) and 15 (31.9%) do not have access to pipe-borne water.

FIGURES 5, 6 AND 7 HOUSING STATUS





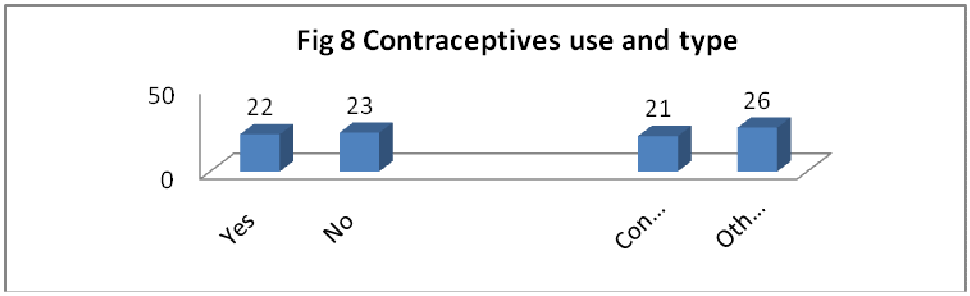


Thirty-nine (83%) have set of radio, 8(17%) do not. Also, 35(74.5%) have a set of television, 12 (25.5%) do not. Thirty-two (68.1%) have access to toilet facility, 15(31.9%) have no access to toilet facility.

Income Status and contraceptive Use

Income level of the respondents predominantly lies between GH¢50.00- GH¢450 per annum, 23 (48.9%) receive income of less than GH¢50.00 annually, 6(12.8%) GH¢50.00- GH¢100.00. Very few receive more than GH¢450.00 per annum.

Twenty-two (46.8%) said they use contraceptives and 23(46.9%) said they do not use any contraceptive, while 2 did not disclose their contraceptive use. Among those interviewed, 12 (22.5%) are not in any relationship 5(10.6%) do not engage in sexual activities, some have lost their partners while others have different views. Twenty-one (44.7%) said they use condom as contraceptive, 26(55.3%) did not reveal the type of contraceptive they use.



DISCUSSION

The educational levels from the fore discussion indicate that most of these HIV patients are enlightened and have, to some extent, knowledge about the disease. This means that there is some negligence or people refusal to adopt the ABC rules regarding the prevention of the HIV which contradicts the findings by (12) who found out that individuals with primary and below level of education are mostly affected with the disease due to lack of higher education, which according them, enhances easy assimilation and understanding of information disseminated.

Because a good number of them are married it may implies most of the transmission of the virus is by heterosexual contacts and not dominated by any means such as use of infested tools, blood transfusion and others. This augment the study by (13) who found from the data gathered from Nasarawa State of Nigeria that 60.3% of the patients diagnosed with HIV/AIDS are married people. Akanni et al (14) confirmed it from a study on socio-demographic characteristics of young adults screened for HIV in a Tertiary Health Centre in Southern Nigeria. This may also show that most of them are unfaithful and or refuse to use protective devices such as condom to prevent the spread of the disease.

Though the data was collected in Kumasi and Accra which are the two main cities in Ghana, majority of them are mainly Akans probably because they form the dorminant tribe in Ghana. Also most of them are Christians because of the predominance of the Christian religion in Ghana, more especially in the Southern Ghana where the subjects were drawn from.

Majority being in the unemployed zone contributes immensely towards the spread of the disease since some of these people adopt immoral means by practicing prostitution in order to make ends meet. This agrees with (13) research showing that most of the people who are housewives and the unemployed contract the virus.

Also most of them receive support from friends and relatives which correlates positively to the level of unemployment to some extent since those who do not work have to rely on their relatives and friends for some support. Others receive support from some NGOs and government agencies.

In Ghana tenancy is common and most average Ghanaians rent flat to live in which is also connected to the national grid. Those who don't enjoy everyday supply of power may be as a result of failure to pay bills or are not connected at all. But there are few of such situations in Ghana, especially in Accra and Kumasi.. . In terms of water, most of them drink pipe-borne water. Those without access to this treated water depend on water from other sources such as well, river, stream and others. Though pipe-borne water is known to be the best, water from deep well are equally good for drinking and for other household chores.

Lack of access to toilet facility is a very crucial issue since it is common in some parts of the country where people queue to attend to places of convenience such as the public toilets. People join long queues and spend a long time to attend to nature's call. This is uncalled for and should be avoided completely.

Due to very low income level which was displayed by these people, it clearly shows that the disease is dominant in the so called poor people who are mostly unemployed and have to depend on their relatives and others means for support.

CONCLUSION AND RECOMMENDATIONS

The results show a that greater number of the subjects are females with a good number of them lying below the age 40 years with at least 10 years of education but surprisingly low levels of income because of lack of jobs. It can therefore be inferred that the number of the people that contract the disease will probably reduce should there be an avenue for employment and other in-built structures put in place for the youths in these regions

Based on the above, the following suggestions and recommendations are made:

Promoting abstinence and faithfulness, promoting reductions in the number of sexual partners, encouraging delays in the onset of sexual activity among adolescents, promoting the correct use and consistent availability of condoms; strengthening

programmes for STD control and encouraging voluntary counselling and testing.

One set of intervention focuses on encouraging people to abstain from sex before marriage and remain faithful to a single partner. This could be promoted through a combination of mass media, counselling, and education programmes. Delays in the onset of sexual activity among adolescents can have a significant impact on the spread of HIV. Information, education, and interventions to limit sexual transmission, encouraging voluntary counselling and testing communication and other programmes that address adolescents and the needs of young people.

The reduction in the number of men who have unprotected sexual contact with prostitutes and bar attendants would contribute immensely in bringing the epidemic under control.

Also promotion of condom use through mass media, counselling and education and to increase the availability of condoms through expanded public distribution, social marketing programmes, and programmes in the workplace. Special initiatives to promote condom use among high-risk populations such as commercial sex workers and long-distance truck drivers have proven effective in some countries. Recent efforts to increase risk perception especially among young people are, however, yielding some results as can be seen from the increased sales of condoms since the launch of the "STOP AIDS LOVE LIFE" in 2000 campaign.

Another intervention focuses on controlling the spread of STDs such as syphilis, gonorrhoea and chanchroid because of the high positive correlation between HIV and sexually transmitted infections (STIs). Most individuals with STIs do not seek appropriate care for example a recent study in Mwanza, Tanzania, found that an improved STD prevention and treatment programme was associated with a reduction of 42% in the number of new HIV infections (1).

One of the critical areas for the reduction of HIV infection is through programmes targeted at the prevention of mother-to-child transmission (MTCT). Various approaches can be used to reduce the number of children who are infected. Interventions such as counselling, medical management, counselling on feeding options and the provision of (an anti-retroviral drug) Nevirapine.

Health officials need to continue efforts to avoid infection through blood transfusion by keeping the blood supply to patients as safe as possible. This can be done by screening blood through laboratory tests and screening potential blood donors through interviews to reject as donors those who have a high probability of infection.

Each of the measures described above can make an important contribution to the reduction of HIV. Adoption of these measures in isolation is not likely to solve the problem completely; some people will respond to or be affected by one type of intervention while others will respond to or be affected by another. Computer simulations suggest that a much larger effect can be achieved by implementing all the interventions together in a broad attack on the epidemic. An effective blood-screening programme, represented by the second line from the top reduces prevalence only modestly. However, an effective STD control programme brings expected prevalence down by about 12%, and condom promotion and partner reduction interventions reduce HIV prevalence even more. Most importantly, when all four interventions are implemented simultaneously, the projected prevalence is reduced.

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REFERENCES

1. National AIDS/STI Control Programme, Disease Control Unit Ministry of Health. (2001) Data File
2. UNAIDS, (2008a). Sub Saharan Africa. Retrieved from: www.unaids.org/en/countryresponses. (Accessed date: September 24, 2009)
3. CDC. (2002) Centers for Disease Control (CDC) and prevention. HIV/AIDS Surveillance Report, 13(2): 1-44.
4. Giri, T.K., J.P. Wali, H.S. Meena, I. Pande, S. Uppal and S. Kailashi, (1995). Socio demographic characteristics of HIV infection in Northern India. J. Comm. Dis., 27(1): 1-9.
5. Cheong, I., A. Lim, C . Lee, Z. Ibrahim and K. Sarvanathan (1997). Epidemiology and clinical characteristics of HIV-infected patients in Kuala Lumpur. Med. J. Malays., 52: 313-317.
6. Laah, J.G. (2003). The prevalence of HIV/AIDS in Zaria, Kaduna State. J. Popul. Assoc. Nigeria. 3(1): 95-101.
7. Mamman, M., (2003) Gender HIV-infection and AIDS - Related deaths in sub-Saharan Africa. J. Popul. Assoc. Nigeria, 3(1): 79-94.
8. Federal Ministry of Health (FMoH), 2001/2005. HIV/syphilis sentinel seroprevalence survey in Nigeria. NACA/FMoH, Abuja.
9. Mamman, M. (2006) Factors fuelling HIV/AIDS in sub-Saharan Africa. Being a paper presented at a HIV/AIDS workshop with long distance truck drivers in the North West Zone of Nigeria, Organized by Partnership for Life, 12 September 2004 at Local Government Service Commission, Muhammadu Buhari Road, Kaduna.
10. National Population Commission and ICF Macro, (2009). Nigeria Demographic and Health Survey 2008. Abuja.
11. Ghana Statistical Service. (2000) *Population Census of Ghana: Preliminary Report.*Accra.
12. Odimayo, M. S, Adediran, S.O. and M.A. Araoye. (2010) Socio-Demographic Characteristics of Adults Screened for HIV/AIDS in a Rural Community in Benue State, Nigeria
13. Laah, J.G. and E. Ayiwulu. (2010) Socio-Demographic Characteristics of Patients Diagnosed with HIV/AIDS in Nasarawa Eggon
14. Akanni CI, O Erhabor. (2005) Socio-demographic Characteristics of young adults screened for HIV in a Tertiary Health Centre in Southern Nigeria. *Highland Medical Research Journal*.; **3**(1): 24-30.

ORIGINAL ARTICLE

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PATTERN OF MICROBIAL COLONIZATION OF THE VAGINA OF DIABETICS IN IBADAN, NIGERIA

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ABSTRACT

The pattern of microbial flora of the vagina of diabetics was studied, to advise on empirical regimen for the treatment of sepsis in diabetics with the lower genital tract as source. In 2003, microscopy, culture and sensitivity of high vaginal swabs from 60 consecutive non-insulin-dependent diabetics and 20 non-diabetics attending Oluyoro Catholic Hospital, Ibadan were done. The fasting plasma glucose was estimated.

The prevalence of micro-organisms decreased with duration of diabetes. The isolates were *Gardnerella vaginalis, Candida* species, *Staphylococcus epidermidis*, *Klebsiella* species, *Enterococcus faecalis*, and viridans Streptococcus. *Klebsiella* species and *Escherichia coli* were incriminated in Gram negative bacilli (GNB) bacteraemia in diabetics in this environment.

In addition to metronidazole, ceftazidime or ceftriazone should be used as first line drugs, while the quinolones should be reserved for the treatment of sepsis in diabetics where lower genital tract is the likely source of infection.

KEYWORDS: Microbes, vagina, diabetics.

INTRODUCTION

Immediately post-delivery, circulating maternal oestrogen may result in microbial flora in babies, similar to that in adults. This changes in approximately two weeks as oestrogens are metabolised, so that throughout childhood the vaginal epithelium lacks glycogen, and so carries a scanty background flora of skin organisms and upper respiratory tract commensals [1]. The post-pubertal vaginal epithelium has a high glycogen concentration due to the influence of circulating oestrogens. This is metabolised by lactobacilli to lactic acid, producing a low pH. The vaginal flora may superficially resemble faecal flora due to the transient peri-anal contamination, but many organisms have a very close association with the lower genital tract e.g. *Bacteroides bivius (*now *Prevotella bivia), Gardnerella vaginalis* and *Mycoplasma hominis*[1]. In vaginal secretions, anaerobes outnumber aerobes by 10^9 to 10^8per ml. The commonest anaerobes found are lactobacilli, *Bacteroides* spp. and anaerobic Gram-positive cocci. Diphtheroids and coagulase-negative Staphylococci are the commonest aerobic bacteria[1]. *Staphylococcus aureus* is recovered from the vagina of only about 5% of healthy women[4]. Yeasts are carried by about 15 –

20% of healthy women[4]. With the onset of sexual activity, statistically significant increases are observed in the prevalence of *Gardnerella vaginalis*, lactobacilli, Mycoplasma and Ureaplasma, but the prevalence of group B Streptococci, *Stapylococcus aureus* and yeasts are not significantly altered[6].

Although our descriptive knowledge of vaginal microbiology has increased, our understanding of the factors controlling the flora remains primitive[7]. Specific and non-specific vaginal host defenses have been catalogued but again, their precise significance is unclear[8].

Diabetes mellitus is a constellation of abnormalities caused by a relative or absolute lack of insulin[9]. It is a state of chronic hyperglycaemia[10]. A random venous plasma glucose of 11.1 mmol/l or more on two occasions or a fasting value of 6.7 mmol/l or more on two occasions is diagnostic[10,11,12]. In the temperate regions, there are two common types of Diabetes mellitus. Type 1 (insulin dependent) and type 2 (non-insulin dependent) accounting for about 15% and 85% of cases respectively. These also occur in the tropics. A third type of the disease seen in the tropical countries has been termed variously as J-type, Z-type, type III or tropical pancreatic diabetes. Such cases are often associated with malnutrition. Some term this as malnutrition–related diabetes mellitus. Type II diabetes is by far the more common of the two main types of the disease[13].

Diabetics are more susceptible to infection than non-diabetics because of the immune response resulting from combined factors such as increased glucose content of blood and tissue, impairment of chemotaxis, serum opsonisation and phagocytosis and lower capacity of tissue reaction to antigenic stimuli[14]. Numerous localized infections are often accompanied by transient phase of bacteraemia[15], some common infections in diabetics include urinary tract infections, gram-negative pneumonia, malignant otitis externa, acute pyelonephritis, diabetic foot, tuberculosis and acute cholecystitis[16]. The lower genital flora may act as the endogenous source of infection that occurs in diabetics.

We studied the pattern of microbial flora of the vagina of diabetics without symptoms and signs of genital tract infection, and their antibiotics sensitivities to advise on empirical regimen for sepsis in diabetics with the lower genital tract as the likely source.

PATIENTS, MATERIALS AND METHODS.
From January to April 2003, consenting 60 consecutive known non – insulin-dependent diabetic and 20 non-diabetic women attending Oluyoro Catholic Hospital, Ibadan were studied. Intake of either an antibiotic or anti-fungal agents within the previous two months and the presence of symptoms and signs of genital tract infections were exclusion criteria.

The age, duration of diabetes, type of anti-diabetic drug, history of vaginal discharge and itching were recorded. Clinical examination for features of lower genito-urinary tract infection was done. High vaginal swab (HVS) was collected with sterile cotton-tipped applicator for microscopy, culture and sensitivity. The pH of the vaginal secretion was done by using a narrow range pH paper, the Potassium hydroxide (KOH), whiff test, was done. Two mls of venous blood was collected from each participant for fasting plasma glucose (FPG) estimation.

Laboratory Procedures
Wet smear microscopy of HVS was prepared for detecting inflammatory cells, motile trichomonads, yeast cell and characteristic "clue" cells. Inoculation was made on Blood, MacConkey and Chocolate agars. The Blood and MacConkey plates were incubated aerobically,while Chocolate plates were in candle extension jar, at 37^{o}C overnight. All yeast and bacterial isolates were identified by conventional laboratory methods[10]. The anti-biotic sensitivities were determined by using Stoke's disc diffusion technique[17]. FPG estimation was done according to standard method[18].

RESULTS

Most diabetics (26.7%) were within age range 21 – 30 and 41 – 50(Table 1, Panel 1). The patient's FPG ranged from a mean of 6.8 – 11.1 to 22.3-27.8mmol/L, with the control group having 6.7mmol/L (Table I, panel 2). At the mean FPG of 8.4mmol/L, the isolates gave a prevalence of 45%, while at 13.9mmol/L and 25.1mmol/L the percentages were 90% and 100% respectively, a statistically significant finding ($p<0.00001$). Greatest isolates were from the elderly diabetics (28.4% of 51-60 years), then from those in the age range of 31-40 years (24.3%).

Forty-five percent of diabetics (27 out of 60) were not sure of the duration of their ailment, 41.9% of the isolates were from this group. With increasing duration of diabetes, there was a decrease in the yield of isolates (24.3%, 18.9%, 10.9% and 4.1%) with a statistically significant finding ($p<0.00001$) (Table I, panel 3). Twenty-two (36.6%) of the patients had 'clue cells' on wet preparation, while none of the controls had, a statistically significant finding ($p= 0.0008$). A total of 74 microbial isolates were from the 60 patients, while 12 were from the 20 controls. The isolates are as shown in table II. Out of the 74 microbial isolates, 54 were bacteria and 20 were fungi; 18 *Candida albicans* and 2 *Candida tropica`lis* The ratio of Gram negative to Gram positive was 1.0:1.5.

The Gram-negative bacteria were *Escherichia coli* (21.6%) and *Klesiella* species (8.1%), while the Gram positive cocci were *Staphylococcus auerus* (24.3%), *Staphylococcus epidermidis* (9.5%), *Enterococcus faecalis* (5.4%) and viridans Streptococci (4.1%). The fungi were *Candida albicans* (24.3%) and *Candida tropicalis* (2.7%). Ten percent of the participants had multiple agents. Augumentin, gentimicin and cefuroxime were active against an average of half of the bacterial isolates, over two-third of the isolates were sensitive to ceftazidime and ceftriazone while the quinolones (ofloxacin and ciprofloxacin) were active against 78 – 100% of the bacterial isolates.

DISCUSSION

In this study, the yield in microbial isolates from the vagina of diabetic decreased with increasing duration of the ailment. This indicates that the diabetics with longer duration of ailment on oral hypoglycaemic agents would have achieved and maintained euglycaemic control, a situation required for control of infection in diabetics. This is explained further by the statement that secondary abnormalities in leukocyte function (chemotaxis, phagocytosis, and intracellular killing) related to glycemic control may contribute to poor host responses to fungal infections[19]; and other infections. Hence emphasis should be placed on timely and adequate control of diabetes mellitus to prevent infection of the lower genital tract and complications such as bacteraemia and sepsis syndromes. The antibiotic sensitivity pattern of the bacterial isolates is shown in table III. Co-trimoxazole, tetracycline and amoxycillin were active against about a third of the bacterial isolates.

TABLE I: AGE, FASTING PLASMA GLUCOSE, DURATION OF DIABETES AND MICROBIAL ISOLATES FROM RESPONDENTS

PANEL 1: AGE OF RESPONDENTS AND MICROBIAL ISOLATES

AGE RANGE (YEARS)		AGE DISTRIBUTION		DISTRIBUTION OF ISOLATES	
		Number	% Distribution	Number	% Distribution
11 – 20		2	3.3	4	5.4
21 – 30		16	26.7	15	20.3
31 – 40		12	20.0	18	24.3
41 – 50		16	26.7	16	21.6
51 – 60		14	23.3	21	28.4
TOTAL		60	100.0	74	100.0

PANEL 2: FASTING PLASMA GLUCOSE (FPG) OF RESPONDENTS AND MICROBIAL ISOLATES

FPG (mmol/L)		FPG DISTRIBUTION		DISTRIBUTION OF ISOLATES	
		Number	% Distribution	Number	% Distribution
6.8 – 11.1		22	36.7	23	31.1
11.2 – 16.6		20	33.3	26	35.1
16.7 – 22.2		16	26.7	20	27.0
22.3 – 27.8		2	3.3	5	6.8
TOTAL		60	100	74	100
(CONTROL)		20		12	

PANEL 3: DURATION OF DAIBETES AND MICROBIAL ISOLATES

DURATION (MONTHS)		DURATION DISTRIBUTION		DISTRIBUTION OF ISOLATES	
		Number	% Distribution	Number	% Distribution
0 – 12		18	30.0	18	24.3
13 – 24		8	13.3	14	18.9
25 – 36		5	8.3	8	10.8
37 – 48		2	3.3	3	4.1
Unknown		27	45.0	31	41.9
TOTAL		60	100.0	74	100.0

From puberty to menopause, the commensals of the vagina include lactobacilli, anaerobic or microaerophilic Streptococci, *Clostridium* species, Bacteroides, *Acinetobacter* species, Fusobacteria. *Gardnerella vaginalis*, Mycoplasma and small numbers of Diphtheroids and Yeasts. After menopause, Diphtheroids, Micrococci, *Staphylococcus epidermidis*, viridans Streptococci, Enterobacteria, *Candida albicans* and other Yeasts[1,10] inhabit the vagina. However, in this study, the resident organisms in the vagina of diabetics, age range 15.5 to 55.5 years, were *Escherichia coli*, *Klebsiella* species, *Staphylococcus auerus, Staphylococcus epidermidis, Enterococcus* faecalis, viridans Streptococci and *Gardnerella vaginalis*, a finding resembling the commensals in post–menopausal women. Whereas it is expected that the flora should consist of microbes found in childbearing adults.

TABLE II: MICROBIAL ISOLATES FROM RESPONDENTS

PANEL 1 : SINGLE ISOLATES

ISOLATES	**FREQUENCY**	**%**
Candida albicans	**18**	**24.3**
Staphylococcus aureus	**18**	**24.3**
Escherichia coli	**16**	**21.6**
Staphylococcus epidermidis	**7**	**9.5**
***Klebsiella* species**	**6**	**8.1**
Enterococcus faecalis	**4**	**5.4**
Viridans Streptococcus	**3**	**4.1**
Candida tropicalis	**2**	**2.7**
Total	**74**	**100.0**

PANEL 2 : MULTIPLE ISOLATES

ISOLATES	**FREQUENCY**	
Klebsiella spp. & E. coli	**2**	
Staphylococcus aureus & E. coli	**2**	
Staphylococcus aureus, Enterococcus faecalis & Candida albicans	**2**	
	6 out of 60 participants	**10**

It is probable that the high glucose content of the tissues favours the growth of this type of flora, and therefore may be one of the factors controlling the flora of the vagina. Further study is required to compare the microbial flora in post-menopausal diabetics in this environment. Candida species was the most prevalent organism of the isolates from the vagina, being 27% (*Candida albicans* 24.3% and Candida *tropicalis* 2.7%). In diabetics, there is a measurable increased rate of bacterial infections in soft tissues secondary to diabetic vascular

disease/neuropathy. Exposure to antibiotics and poor vascular supply can play an important part in the incidence of fungal infections of the soft tissues[19]. Although most of the cases of vaginal candidiasis in diabetics are caused by *Candida albicans*, this group of patients may have a slightly higher incidence of *Candida glabrata* as an aetiological agent[19]. However in our study, *Candida glabrata* was not found.

Of the 54 bacterial isolates, the Gram-negative bacilli to Gram-positive cocci ratio were 1:1.5. The Gram-positive cocci were *Staphylococcus auerus* 24.3%, *Enterococcus faecalis* 5.4% and viridans Streptococci 4.0%. It is pertinent to note that the main Gram-negative were *E. coli* and *Klebsiella* species. These findings are important in view of the fact that *E. coli* has been reported as the leading agents of Gram-negative bacilli bacteraemia [20], and in this environment, it was found to be the second leading cause of bacteraemia in diabetics[20]. The lower genital tract should be considered as an important source of Gram-negative bacilli in diabetics with bacteraemia or sepsis.

Ten of the patients had multiple microorganisms. Two (3.3%) each had *Klebsiella* species and *E. coli; Staphylococcus auerus* and *E. coli*; and one had three, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*. We did not culture for anaerobes because of technical reasons, however 22 (36.6%) of the patients had 'Clue cells' on wet preparation while none of the controls had. This finding is statistically significant. This indicates that these women were habouring anaerobes, and that the hyperglycaemic state facilitates the growth of *Gardnerella vaginalis* and anaerobes in the vagina.

There was a picture of multidrug resistance by the bacteria to most of the commonly used antibiotics. Co-trimoxazole had <10% to each of the organism, streptomycin invariably recorded 0% while tetracycline and amoxycillin had similar picture. This is a similar pattern to the reported antibiotic sensitivity pattern of isolates from blood of diabetics in the same environment [20]. Augmentin, gentimicin and cefuroxime were active against about half of the bacteria, ceftazidime and ceftriazone against two-third, while most bacteria had good sensitivity to the quinolones (ofloxacin and ciprofloxacin) (78.3-100%). The lower activity of cefuroxime may be as a result of fact that it has been introduced into this environment long before the third generation cephalosporins.

Early antibiotic treatment reduces mortality. It has been documented that delay in introduction of appropriate antibiotics was associated with significant increase in hospital stay and with development of acute organ failure[21]. Therefore, we suggest a timely onset of empirical antibiotics in diabetics with sepsis, whose lower genital tract is the likely source.

The cephalosporins, (ceftazidime or ceftriazone) should be used as first line drug, while the quinolone (ofloxacin or ciprofloxacin) should be used as reserved drugs, to prevent resistance. In view of the fact that 36.6% of these women harbour *Gardnerella vaginalis* and possibly anaerobes, metronidazole should be combined with the antibiotics. This regimen should continue until the substantive antibiotic sensitivity results are available and/or a change of regimen is desirable. In addition, it is important in treatment to achieve and maintain euglycaemic control, because response to therapy can be correlated with glycaemic control[19].

REFERENCES:

1. **P. M. Hawkey and D. A. Lewis. Medical bacteriology. A practical approach. IRL Press at Oxford University Press. 1989. Page 71.**
2. **Tashjian J. H. Coulman C. B. and Washinton J.A. Vaginal flora in asmptomatic women. Mayo. Clin. 1976. Proc.551-557.**
3. **Easmon C. S. F. and Ison C. A. Gardnerella vaginalis. Lancet 1983. 2: 343.**
4. **Sobel J.D. Epidemiology and pathogenesis of recurrent vulvo-vaginal candidiasis. Am. J. Obstet.gynaecol. 1985. 151: 924-935.**
5. **Brown W. J. Variations in the bacterial flora. A preliminary report. Am. Inter. Med. 1982. 96: 131.**
6. **Sobel J.D. Epidemiology and pathogenesis of recurrent vulvo-vaginal candidiasis. Am. J. Obstet gynaecol. 1985. 151: 924-935.**
7. **Shafer M. A., Sweet R. L. and Ohun-Smith M. J. Microbiology of the lower genital tract in post-menarchal adolescent girls: differences in sexual activity, concentration and presence of**

non specific vaginitis. J. Pedaitr. 1985. 107: 944-981.
8. Hill G. B., Eschenbach D. A. and Holme K. K Bacteriology of the vagina. Scand. J. virol. Nephrol 1984. 18 (suppl. 86): 23-80.
9. Cohen M. S., Balsk J. R. and Proctor R. A. Host defenses and the vaginal mucosa. A revelation. Scand. J. virol. Nephrol 1984. 8 (suppl. 86): 13 – 22.
10. Ganong W. F. Review of Medical Physiology 13th Edition. Lange Medical Publication. 1987. Page 251.
11. Moinca Cheesbrough. Manual of Medical Laboratory Sciences. ELSS. 1993; Reprint: Vol. 1: 114, Vol. II : 248 – 272.).
12. WHO expert Committee on Diabetes Mellitus. Impaired glucose tolerance and diabetes mellitus. WHO Criteria. Brit. Med. J. 1980; 281: 1512-1513.
13. Alberti KGMM. The diagnosis of diabetes: problems and perspective (Editorial). Int. Diabetes Digest. 1992. 3(3): 65-66.
14. McCance D. R. , Hanson R. L. , Charles M. A. , Jacobson L. T. H. , Pettit D. J., Bernett P. H., Knowler W. C.. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diabetic methods for diabete. BMJ. 1994; 308: 1323-1328.
15. Odugbesan O. and Barnett A. H. Racial differences In Barnett A. H. (ed). Immunogenetics of insulin dependent diabetes. Lancaster. MTP Press 1987. pp 91-101.
16. Robertson H. D. Polk H. C.(Jr.). The Mechanism of Infection in patients with diabetes mellitus. A review of leukocyte malfunction. Surgery. 1974; 75(1): 123-128.
17. Balows A., Davies B. I. and Vandepitte J. WHO Bench level Manual of basic Bacteriology 1985.
18. A. A. Oni, M. O. Ogunkunle, A. A. Oke and R. A. Bakare.Pattern of gram negative rods bacteraemia in diabetic patients in Ibadan, Nigeria. 2000. Afr. J. Med. med. Sci. 29: 207-210.
19. John R Perfect MD and Gary M Cox MD Fungal Infections in Diabetic Patients and Patients with Renal Failure (2001) Current Treatment Options in Infectious Diseases 2001, 3:523-532
20. Mandel G. R., Douglas R. C. Bennettee J. E. Principle and Practice of Infectious Diseases. 3rd Edition. Wiley Medical Publication. 1990: 611-636.

TABLE III: ANTIBIOTIC SENSITIVITY PATTERN OF THE ISOLATES

Isolates	No	A N T I B I O T I C S											
		Ceftazidime	Ceftriazone	Cefuroxime	Ofloxacin	Ciprofloxacin	Augumentin.	Gentamycin	Tetracycline	Amoxycillin.	Co-trimoxazole.	Sreptomycin.	Erythromycin.
E. coli	16	87	74	60	88	85	50	63	25	38	6	0	NT
Klebsiella spp	6	85	70	58	83	82	47	56	20	38	7	0	NT
Staph. aureus	18	86	78	44	83	82	48	56	23	40	7	0	65
Staph. epidermidis	7	82	71	43	78	80	48	57	18	40	6	0	58
Enterococcus faecalis	4	99	83	59	89	86	38	60	30	38	5	0	55
Viridans Streptococci	3	100	89	61	90	100	68	60	32	42	10	5	70

NT: Not tested

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EFFICACY OF THREE DISINFECTANT FORMULATIONS AGAINST MULTIDRUG RESISTANT NOSOCOMIAL AGENTS

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RUNNING TITLE: DISINFECTANT FORMULATIONS AGAINST MULTIDRUG RESISTANT NOSOCOMIAL AGENTS

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ABSTRACT

The current increase in the prevalence of nosocomial infections within the hospital environment despite adequate cleaning and disinfection can be said to be due to the following: (i) ineffectiveness of the various disinfectant formulations used in infection control on the various hospital equipments and wards; (ii) development of resistance to the various chemical disinfectant formulations been used in the hospitals by the various microorganisms.

Ten bacteria isolates from different clinical specimens of hospitalized patients identified using standard bacteriological methods and found after screening to be resistant to two or more classes of the antibiotics: cephalosporins, quinolones, Betalactams, nitrofuran, macrolide and an aminoglycoside using the Kirby-Bauer method of disc diffusion test were used in this study. These were subjected to susceptibility testing against three selected disinfectant formulations (Izal, Dettol and Jik) at the manufacturer's dilutions and half the dilutions prescribed on their labels by using agar diffusion method. Out of these clinical isolates used in this study, 5 (50%) and 2 (20%) were resistant to manufacturer's dilution and half the dilution of Dettol respectively, 9 (90%) and 6 (60%) to manufacturer's dilution and half the dilution of Izal respectively while 1 (10%) and 0 (0%) were resistant to manufacturer's dilution and half the dilution of Jik respectively.

The resistance demonstrated by some of the nosocomial agents in this study against the selected disinfectant formulations at their manufacturer's dilution and half the prescribed dilutions showed the probability of the nosocomial agents developing some mechanisms of resistance against the various disinfectant formulations rather than ineffectiveness of the disinfectant formulations. However, the effectiveness of Jik formulation at half the manufacturer's prescribed dilution shows that Jik is still an important disinfectant formulation in the control of nosocomial agent most especially the resistant strains.

INTRODUCTION

The term disinfectant is generally used for chemical agents employed to disinfect inanimate objects. They are use to reduce or eliminate pathogenic microbes in or on materials so that they are no longer a hazard. The process of using chemical disinfectants in inhibiting or killing pathogenic microorganisms is known as disinfection. Disinfection is however not an absolute term, this implies that some living microbes may persist but the possibility of sterilization resulting from disinfection cannot be ruled out (1, 22). Disinfectants used to treat skin and other external body membranes and cavities are termed Antiseptics (1, 22).

There are various classes of chemical disinfectants, these are; Acids and their esters, Alcohols, Aldehydes, Biguanides, Halogens, Heavy metals, Oxidizing compounds, Phenols and Phenolic compounds, Surface active agents, Quinoline and Isoquinoline derivatives and Dyes (1, 22). The choice of the most appropriate disinfectant formulation for a particular purpose depends on some factors like (1):

- Properties of the chemical agent in terms of activity and solubility
- Microbiological challenge in terms of types and level of microbial contamination
- Intended application either for antiseptic or disinfection
- Environmental factors such as presence of organic matter or specificity of microorganism to a particular environment
- Toxicity of the agent i.e disinfectant toxicity effect.

In the hospitals, disinfectants have been found to play an important role in the prevention and control of hospital acquired infections (Nosocomial infections). Nosocomial infections are those infections acquired in hospital or healthcare service unit that first appear 48 hours or more

after hospital admission or within 30 days after discharge following in-patient care (2).
They are unrelated to the original illness that brings patients to the hospital and neither present nor incubating as at the time of admission (2, 3). Nosocomial agents could be bacteria, fungi, viruses and protozoan in nature (4). There are several reasons why nosocomial infections are more alarming than the community acquired infections; firstly, many medical procedures that bypass the body's natural protective barriers could result into infecting the patients. Secondly, medical staffs move from patient to patient thus providing a way for pathogens to spread. Thirdly, inadequate sanitation protocols regarding uniforms, equipment sterilization, washing with disinfectant and other preventive measures that may either be unheeded by hospital personnel or too late to sufficiently isolate patients from infectious agents and lastly the routine use of antimicrobial agents especially the broad-spectrum antibiotics in hospitals creates selection pressure for the emergence of the resistant strains of microorganisms [(2)].

The significance of nosocomial infection lies not only in its ability to substantially alter morbidity and mortality statistics, but also in its economic implications [(5,6)]. Nosocomial infection prolongs duration of hospitalization, increases the cost of health care, emergence of multiple antibiotic resistance microorganisms and reduces the chances of treatment for others (7, 8, 9).

In various hospitals, one of the ways among others, of controlling spread of infections within the hospital environment is the use of chemical disinfectant formulations in disinfecting the hospital environment and equipment, washing of hands when moving from patient to patient and after and disinfection of patient's skin before injection, catheterization and operation is perform on them (19). However, failures in the antimicrobial activity of some of the disinfectants have been reported (1, 22). The utilization of phenolic constituent of some phenolic disinfectant as carbon source by some bacteria such as *Pseudomonas aeruginosa* and complete resistance of some microorganisms to some of the classes of disinfectants e.g. *Staphylococcus aureus*, are some of these reports (1, 22). The possibility of more microorganisms particularly the nosocomial bacteria further developing resistance to more of these disinfectant formulations, just like they do with antibiotics, underscore the need to constantly evaluate the antimicrobial activities of various chemical disinfectant formulations against the nosocomial agents.

This study therefore, assesses the efficacy of some disinfectant formulations in Nigerian markets at their prescribed dilutions/concentrations of use by their manufacturers against some multidrug resistant bacteria of nosocomial origin.

MATERIALS AND METHODOLOGY

BACTERIOLOGY

The microorganisms used in this study were obtained on slants as pure culture from the microbiology unit of the University College Hospital (UCH) Ibadan, Oyo State from hospitalized patients who develop infection diagnosed to be acquired from the hospital environment. Ten microorganisms mainly bacteria were collected and used in this study. They include: Two strains of *Pseudomonas aeruginosa* (P1 and P2), Two strains of *Staphylococcus aureus* (S1 and S2) Two strains of *Klebsiella* species (K1 and K2), Two strains of *Proteus* species (Pr1 and Pr2) and Two strains of *Escherichia coli* (E1 and E2).

DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY PROFILES OF THE TEST CLINICAL ISOLATES USING STANDARD ANTIBIOTIC DISCS

The standard disk diffusion method recommended by the National Committee for clinical laboratory standards (NCCLS, 2003) was used in determining bacterial susceptibility to antimicrobials as described by Qin et al in 2004. The antibiotics used are Gentamicin, Cefixime, Ofloxacin, Ceftazidime, Augmentin, Nitrofurantoin, Ciprofloxacin, Cefuroxime, Erythromycin, Cloxacillin and Ceftriaxone. The standard antibiotic discs were placed at equal distance in a circular pattern on the surface of the Mueller Hinton agar with the aid of a sterile forcep. The antibiotic discs were designated differently for Gram positive and Gram negative organisms. The plates were then incubated at $37^{o}C$ for 24hrs in an upside down position. The zones of growth inhibition were then recorded.

PREPARATION OF THE TEST CONCENTRATIONS OF DISINFECTANT FORMULATIONS

Two test dilutions each; of the three disinfectant formulations were prepared at a dilution a little below the dilutions prescribed for use by the manufacturers (e.i. higher concentrations). They were diluted to the test concentrations with sterile distilled water as stated below.

- DETTOL (3%v/v and 6%v/v)
- IZAL (0.5%v/v and 1%v/v)
- JIK(SODIUM HYPOCHLORITE) (2.5%v/v and 5%v/v)

ANTIMICROBIAL SCREENING OF THE DISINFECTANT FORMULATIONS

Using agar-cup diffusion method, two dilutions each, of the three test disinfectant formulations was used in this screening. Twenty millilitres of melted and cool Mueller Hinton agar was seeded with 0.2ml of 10^{-2} dilution from an overnight broth culture of the multidrug resistant strains of the test clinical isolates, rolled between palms and poured into sterile petri-dishes and allowed to set. The surface was then dried in a sterile drier and with the aid of a sterile 8mm cup borer; five wells were bored into the agar plates. The first three wells were filled with two drops of the manufacturer's dilutions for the three disinfectant formulations (3%v/v Dettol, 0.5%v/v Izal and 2.5%v/v Jik) and 10µg of Gentamicin was introduced into the fourth well as positive control while sterile distilled water was introduced into the fifth well as negative control. This procedure was also carried out for the corresponding half dilutions of the test disinfectant formulations (6%v/v Dettol, 1%v/v Izal and 5%v/v).

This whole process was done in duplicates. The plates after about one hour of pre-diffusion were then incubated at 37^{o}C for 24hrs in an upright position. The averages of the corresponding zones of growth inhibition were then recorded for both dilutions.

RESULTS

The clinical isolates screened with some antibiotics namely Gentamicin, Cefixime, Ofloxacin, Ceftazidime, Augmentin, Nitrofurantoin, Ciprofloxacin, Cefuroxime, Erythromycin, Cloxacillin and Ceftriaxone shows that they were resistant to more than one class of the antibiotics used, making them multidrug resistant clinical strains. *Proteus* specie Pr1 was resistant to all the antibiotics except nitrofurantoin, *Klebsiella* specie K2, *Pseudomonas aeruginosa* P2, *Escherichia coli* E1 and *Staphylococcus aureus* S1 are resistant to all the antibiotics. *Proteus* specie Pr2 was susceptible only to ofloxacin, ciprofloxacin, nitrofurantion and cefixime, *Klebsiella* specie K1 to nitrofurantoin and gentamicin, *Pseudomonas aeruginosa* P1 to ofloxacin and ciprofloxacin while *Streptococcus aureus* S2 was susceptible to ofloxacin, ceftazidime, cefuroxime, gentamicin and ceftriaxone (Table 1).

The susceptibility test for the three disinfectant formulations against the test clinical isolates shows that few of the test clinical isolates are resistant to some of the disinfectant formulations at a dilution below (i.e. higher concentration) the manufacturers prescribed dilutions as used in this study. *Proteus* specie Pr1, *Pseudomonas aeruginosa* P1 and *Streptococcus aureus* S2 were resistant to 3%v/v Dettol, 0.5%v/v and 1%v/v Izal. *Streptococcus aureus* S1 was resistant to 2.5%v/v Jik, 3%v/v Dettol and 0.5%v/v Izal while *Klebsiella* specie K1 was resistant to the two dilutions of Dettol and Izal used in this study (Table 2).

TABLE 1: ANTIBIOTIC SUSCEPTIBILITY PROFILE OF THE TEST CLINICAL ISOLATES

Gram negative organisms								
Clinical isolates	**Antibiotics zones of growth inhibition (mm)**							
	CXM	**OFL**	**AUG**	**NIT**	**CPR**	**CAZ**	**CRX**	**GEN**
Pr1	**R**	**R**	**R**	**15**	**R**	**R**	**R**	**R**
Pr2	**17**	**15**	**R**	**12**	**20**	**R**	**R**	**R**
K1	**R**	**R**	**R**	**23**	**R**	**R**	**R**	**14**
K2	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
E1	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
E2	**R**	**R**	**R**	**24**	**R**	**R**	**R**	**15**
P1	**R**	**25**	**R**	**R**	**39**	**R**	**R**	**R**
P2	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Gram positive organisms								
	ERY	**CXC**	**OFL**	**AUG**	**CAZ**	**CRX**	**GEN**	**CTR**
S1	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
S2	**R**	**R**	**30**	**R**	**20**	**15**	**15**	**25**

Key:
CXM- CEFIXIME (5µg); OFL- OFLOXACIN (5µg); AUG- AUGMENTIN (30µg); NIT- NITROFURANTOIN (300µg); CPR- CIPROFLOXACIN (5 µg); CAZ- CEFTAZIDIME (30 µg); CRX- CEFUROXIME (30 µg)-; GEN- GENTAMICIN (10 µg); ERY- ERYTHROMYCIN (5 µg); CXC-CLOXACILLIN (5 µg) ; CTR- CEFTRIAXONE (30 µg), R – Resistant.

TABLE 2: ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF THE TEST DISINFECTANT FORMULATIONS AGAINST THE TEST CLINICAL ISOLATES.

Clinical isolates	Detol 1 (3%V/V)	Detol 2 (6%V/V)	Izal 1 (0.5%V/V)	Izal 2 (1%V/V)	Jik 1(2.5%V/V)	Jik 2 (5%V/V)	Controls	
							+ve	-ve
							G (10µg/ml)	W
Test Disinfectants zones of growth inhibition (mm)								
Pr1	**R**	**13**	**R**	**R**	**20**	**24**	**16**	**-**
Pr2	**18**	**20**	**R**	**R**	**25**	**30**	**16**	**-**
K1	**R**	**R**	**R**	**R**	**15**	**17**	**17**	**-**
K2	**13**	**13**	**R**	**13**	**14**	**17**	**17**	**-**
E1	**19**	**21**	**12**	**15**	**14**	**16**	**15**	**-**
E2	**16**	**16**	**R**	**12**	**14**	**15**	**15**	**-**
P1	**R**	**12**	**R**	**R**	**13**	**15**	**R**	**-**
P2	**14**	**15**	**R**	**R**	**12**	**14**	**16**	**-**
S1	**R**	**15**	**R**	**12**	**R**	**15**	**25**	**-**
S2	**R**	**R**	**R**	**R**	**13**	**14**	**13**	**-**

Keys: E- *E. Coli*; K- *Klebsiella* spp; S- *Staphylococcus aureus*; P- *Pseudomonas aeruginosa*; Pr- *Proteus* spp; G – Genticin; W – Water - No activvity

DISCUSSION

All over the world, nosocomial infection is a recognized public health problem; Surveillance programmes estimate the rate of infection at 5-10% of hospital admissions (10, 11, 12, 13). In Nigeria, nosocomial infection at a rate of 2.7% was reported in Ife (15), while 3.8 % [(14)] was reported in Lagos and 4.2 % in Ilorin [(16)]. These continue to increase yearly. Nosocomial infection rates vary substantially by body site, by type of hospital and by the infection control capabilities of the institution [(17)]. Although viruses, fungi, bacteria and parasites are recognized as sources of nosocomial infections, bacterial agents remain the most commonly recognized cause (18).

The emergent of multidrug resistant hospital acquired bacteria have been reported throughout the world and the mechanisms to which they resist the antimicrobial activity of the various antimicrobial agents particularly antibiotics have also been studied extensively (22). However, such studies have not been adequately done for most of the classes of disinfectants in Nigeria. The development of resistance to the Phenolic class has long been reported (22). Some microorganisms, for example, *Pseudomonas aeruginosa*, have been found to utilize some phenolic compounds as their carbon source (1, 22).

In this study, the presence of multidrug resistance bacteria nosocomial agents was observed as all the clinical isolates used in this study were found to be multidrug resistant (100% prevalence). Resistance was observed with some of the clinical isolates being resistant to the disinfectant formulations at the dilution prescribed by their manufacturers. *Proteus* specie (Pr1), *Klebsiella* specie (K1), *Pseudomonas aeruginosa* (P1), and *Staphylococcus aureus* (S1 and S2) were resistant to 3%v/v Dettol and 0.5%v/v Izal while *Staphylococcus aureus* (S1) was resistant to 2.5%v/v Jik. *Proteus* specie (Pr2), *Klebsiella* specie (K2), *Escherichia coli* (E2) and *Pseudomonas aeruginosa* (P2) were resistant to 0.5%v/v Izal. However, some became susceptible when the disinfectants were diluted at half the prescribed dilution i.e higher

concentration than the prescribed concentrations. *Proteus* specie (Pr1 and Pr2), *Klebsiella* specie (K1), *Pseudomonas aeruginosa* (P1 and P2) and *Staphylococcus aureus* (S2) remained resistant to the Izal formulation when the dilution was lowered to half the prescribed dilution (1%v/v) while *Klebsiella* specie (K1) and *Staphylococcus aureus* (S2) remained resistant to 6%v/v of Dettol formulation.

All the clinical isolates were susceptible to Jik formulation when the dilution was lowered to half that of the prescribed dilution i.e higher concentration of 5%v/v. This shows that Jik disinfectant formulation stand as an effective disinfectant formulation against nosocomial agent particularly the multidrug resistant strains of bacteria when used at a much lower dilution (i.e. higher concentration) to that prescribed by the manufacturer.

REFERENCES

1. Hugo and Russell (2004). Pharmaceutical Microbiology, 7th edition; Stephen P Denyer, Norman A Hodges and Sean P Gorman (editors). Pp 220-232; 285-303. Blackwell publishers, UK.

2. Wikipedia Encyclopedia: http:// en.wikipedia. org / wiki / nosoconial_ infection. Retrieved 9th June, 2009.

3. Horan, T.C., Gaynes, R. P., (2004). Surveillance of nosocomial infections. In: Hospital epidemiology and infection control 3rd ed., Mayhall C.G (ed).Philadelphia Lippincortt. Williams and Wilkins: 1659-1702.

4. World Health Organisation. (2002). Prevention of Hospital Acquired Infections, A Practical Guide. WHO/CDS/CDR/EPH/2002.12

5. Wenzel R. P., (1995). The economics of nosocomial infections. *J Hosp. Infect* 31: 79-87.

6. Plowman (1999). The socio-economic burden of Hospital acquired infections. London, Public Health Laboratory Service and the London School of Hygiene and Tropical Medicine,

7. Ricks, Delthia, (2007). "Germ warfare". Ms. Magazine spring. Pp 43-45.

8. Taiwo, S. S., Onile, B. A., Akanbi, I. I. A. A., (2004). Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates in Ilorin, Nigeria. *Afr.J.Clin.Exper.Microbiol*. 5(2):189-197.

9. Robert, A. W., (2001). Controlling Antimicrobial resistance in hospitals infection control and use of antibiotics. *Emerg Infect Dis*. 7(2): 188-192.

10. Hughes, J. M., Jarvis, W. R., (1985). Epidemiology of nosocomial infections. In Lennets, E. H, Balows, A, Hausler, W. J. Jr., Shadomy, H. J., (ed) manual of clinical microbiology 4th ed. Asm, Washington D.C; Pp99-104.

11. Pechere J. C., (1993). Microbiology of nosocomial infections. *Bull Acad Natl Med*.; 177 (5): 705-715.

12. Alausa, K. O., Onile, B. A., (1984). Epidemiological pattern of bacterial septicaemia at the University College Hospital Ibadan. Nig. *Med. Jour*; 14 (1,2): 55-62

13. Klevens, R. M., Edwards, J. R., Richards, C. L., (2007). Estimating health care-associated infections and deaths in U.S Hospitals. *Public Health Rep*. 122 (2):160-166

14. Kesah, C. N., Egri-Okwaji, M. T. C., Odugbemi, T. O., Iroha, E. O., (1999). Bacteria associated with nosocomial infections and their antimicrobial pattern in pediatric patients in a tertiary health institution. *J Med. Med Sci*.1: 6-13

15. Onipede, A. O., Oluyede, C. O., Aboderin, A. O., (2004). A survey of hospital acquired infections in Obafemi Awolowo University Teaching Hospital, Ile-Ife. *Afri. J. Clin. Experi. Microbiol*. .5(1): 108-118

16. Odimayo, M. S., Nwabuisi, C., Adegboro, B., (2008). Hospital acquired infection in Nigeria. *Tropical Journal of Health Sciences*. 15 (1):49-54

17. Haley Reiv, Schachtman, R. H., (1976). The emergence of infection surveillance and control programmes in US Hospitals: An assessment.

18. Dembri, L., Zervos, M. J., Heirholzer, W. J., (1998). Nosocomial bacterial infections, 3rd ed., Pp.501-528. In E.A Brachman (ed.). Bacterial infection in humans. Plenum Publishing Corporation,New York. N.Y.

19. Samuel, S. O., Kayode, O. O., Musa, O. I., Nwigwe, G. C., Aboderin, A. O., Salami, T. A. T., Taiwo, S. S., (2010). Nosocomial Infections and the Challenges of Control in Developing Countries. *Afr. J. Cln. Exper. Microbiol* 11(2): 102-110

20. Qin, D., Weissman, S. J. and Chestnut, M. F., (2004). Kirby-bauer disc approximation to detect inducible third-generation cephalosporin resistance in Enterobacteriaceae. *Ann. Clin. Microbiol. Antimicrobl*., 3:13-13. DOI: 10.1186/1476-0711-3-13

21. National Committee for Clinical Laboratory Standards. (2003). Methods for dilution and antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Wayne, PA.

22. Gerald McDonnell and A. Denver Russell. (1999). Antiseptics and Disinfectants: Activity, Action, and Resistance. *Clinical microbiology reviews*. Vol. 12, No. 1. P. 147–179.