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MOLECULAR IDENTIFICATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN BENIN-CITY NIGERIA

Obasuyi, O.

Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, P.M.B 1154, Benin-City, Edo State, Nigeria. E-mail: osobex@yahoo.com, osahon.obasuyi@uniben.edu Tel: +2348033492141

ABSTRACT

We use the molecular techniques of PCR and PFGE to identify MRSA from clinical isolates of Staphylococcus aureus causing infections among hospitalized patients in Benin-City, Nigeria. A total of 36 isolates were obtained from the University of Benin Teaching Hospital between July-September, 2007. The MRSA strains were selected according to their phenotypic characteristics (antibiotic-resistant profiles), susceptibility to oxacillin by E-test, and detection of β-lactamase. This was verified by a latex agglutination test for PBP2a production combined with PCR for mecA gene carriage. Four isolates representing 11% were confirmed as MRSA according to the molecular techniques used with two PFGE types (H and L) and one agr type (I). Multi resistance to the various antibiotics used was observed in one of the clones. The isolation of MRSA in health institution indicates that adequate steps in limiting spread are urgently needed. Also, for the first time two MRSA clones according to the PFGE classifications have been identified in Nigeria.

Keywords: methicillin-resistant Staphylococcus aureus, MRSA, PFGE, PCR, molecular techniques.

INTRODUCTION

Since 1961, Methicillin-resistant Staphylococcus aureus (MRSA) strains have assumed increasing importance internationally as a cause of both nosocomial and community-acquired infections (1). A recent United States study found that Infections caused by MRSA result in increased lengths of hospital stay, health care costs, morbidity and mortality when compared to those caused by methicillin-sensitive Staphylococcus aureus (MSSA) (2). Although the presence of MRSA in the Asia-Pacific region has been well recorded, data on the true prevalence of MRSA in the region are limited. Data from Australia have been published and shown that in hospital isolates from the Eastern Seaboard, the percentage of S. aureus that were MRSA remained relatively constant at approximately 30% from 1986 to 1994.

Soon after the first reports of methicillin resistance in S. aureus in 1961, the unusual behaviour of the strains in susceptibility tests were noted. Early reports indicated that the MRSA were heterogenous in their expressions of resistance to β-lactam agents, in that large differences in the degree of resistance were seen among the individual cells in a population (3). Infection control measures, such as placing hospitalized patients colonized or infected with MRSA in contact precautions (the use of gowns and gloves), have been demonstrated to limit the spread of this pathogen (4). The use of surveillance cultures (e.g. anterior nares, axillae and perineum) greatly improves the detection of MRSA colonization compared to clinical cultures alone.

The anterior nares are the most frequent site of MRSA colonization, with a single culture of this site having a sensitivity of approximately 85% (5).

Antimicrobial resistance of S. aureus especially MRSA continues to be a problem for clinicians worldwide. However, few data on the antibiotic susceptibility patterns of S. aureus isolates in South Africa have been reported and the prevalence of MRSA in the KwaZulu-Natal (KZN) province (6). Methicillin resistance in Staphylococcus spp. is primarily mediated by the mecA gene, encoding penicillin-binding protein 2a (PBP2a). This protein has reduced affinity for β-lactam antibiotics. Because the mecA gene is heterogeneously expressed in vitro selective media are necessary to facilitate recovery of MRSA in cultures (7).

The time from culture inoculation to identification of MRSA is typically 48 h, with some methods taking as long as 96 h (8) with sensitivity of any single selective medium ranging between 65 and 100%. Several techniques to shorten the time of identification of MRSA in the laboratory have been developed in the last decade, including slide latex agglutination assays to detect PBP2a, a colorimetric cycling probe assay to directly detect the mecA gene (9), a real-time PCR method to detect the mecA gene (10) in conjunction with S. aureus-specific genome fragments, such as nuc and sa442.

Although data on the prevalence of MRSA infections in Africa are limited, one of the earliest reports in the continent was in South Africa (11). Studies in the 1980s and 1990s on MRSA in South Africa have also been described (12). The recent reports of S. aureus intermediate-resistant to
vancomycin and teicoplanin in Africa (13) also indicate that information on antibiotic resistance is critical for optimal decisions regarding hospital formulary and infection control policies. In addition, characterization of strains is important in understanding the epidemiology of *S.aureus* and evaluating the effectiveness of infection control and antimicrobial prescribing measures (14). The main objective of this study was to use the molecular techniques of Polymerase Chain Reaction (PCR) and Pulsed Field Gel Electrophoresis (PFGE) to identify MRSA from clinical isolates of *S.aureus* causing infections among hospitalized patients in Benin-City, Nigeria.

**MATERIALS AND METHODS**

**Study area** – a total of 36 clinical isolates of *Staphylococcus aureus* were obtained from hospitalized patients at the University of Benin Teaching Hospital, Benin City Nigeria between July-September, 2007. Identification and confirmation of isolates were conducted based on growth and fermentation on mannitol salt agar (MSA), colonial morphology, Gram staining and positive results to both catalase and coagulase tests.

**Identification of MRSA**

The MRSA strains were selected according to their phenotypic characteristics (antibiotic resistant profiles), susceptibility to oxacillin by E-test (AB Biodisk), and detection of β-lactamase. This was verified by a latex agglutination test (bioMerieux) for PBP2a production combined with PCR for *mecA* gene carriage.

**Extraction of chromosomal DNA**

DNA was extracted by suspending portions of four to five colonies in 1ml dH₂O equivalent to 2MF, centrifuged for 10mins at 13000rpm. The supernatant was rejected and the sediment resolved with 100ml lysis buffer (50mM Tris-HCl pH=7.5, 1% Triton X-100, 1mM EDTA pH=8.0) + 0.2 µl proteinase K (1µl/ml from stock 20mg/ml). Initial incubation was done at 56°C for 1 hour followed by 95°C for 10mins. The DNA extracts were stored at -20°C prior to use. PCR for the presence of the gene *lin* was used to test for the quality of the DNA extracts (15).

Classification of MRSA strains was performed by PCRs according to agr groups (16). The presence of toxin genes (*tst, PVL, egl*) was investigated by PCRs using specific primers and programmes (17). Chromosomal DNA extraction of MRSA was performed into agarose disks and clonal types was defined by PFGE of SmaI digests (18, 19).

**Latex agglutination test for PBP2a production**

The test was performed according to the manufacturer's instructions. Briefly, the isolates were subcultured onto blood agar at 37°C for 18 hours to obtain fresh growth. A loopful of cells was suspended in four drops of extraction reagent 1 and was placed in a boiling water bath for 3 minutes. After allowing the suspension to cool to room temperature (approx.10 minutes), one drop of extraction reagent 2 was added and the mixture was vortexed thoroughly. The suspension was then centrifuged at 4500 rpm for 5 minutes. The latex agglutination test was performed with the supernatant, and 50µl of the supernatant was mixed with a drop of sensitized latex. For the negative control, 50 µl of the supernatant was mixed with 1 drop of negative control latex. The samples were mixed for 3 minutes on a shaker and the results were evaluated according to the following plan:

<table>
<thead>
<tr>
<th>Sensitized latex</th>
<th>Control latex</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>MRSA</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>MSSA</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>Undetermined</td>
</tr>
</tbody>
</table>

**RESULTS**

A total of thirty-six *S. aureus* isolates collected from hospitalized patients in Benin-City, Nigeria between July and September 2007 were used in this study. Four isolates representing 11% were confirmed as MRSA (Table 1) according to the molecular techniques used with two PFGE types (H and L) (Figure 1) and one agr type (1). Multi resistance to the various antibiotics used was observed in one of the clones. There was no detection of any toxin genes from the strains (Table 2).

<table>
<thead>
<tr>
<th>Number of <em>S. aureus</em> isolates</th>
<th>MRSA</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>4</td>
<td>11.1</td>
</tr>
</tbody>
</table>

TABLE 1: PREVALENCE OF MRSA AMONG THE CLINICAL ISOLATES OF *S.AUREUS*
TABLE 2: RESULTS OF GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF THE 4 MRSA STRAINS

<table>
<thead>
<tr>
<th>PFGE type</th>
<th>agr</th>
<th>Antibiotic resistance</th>
<th>Toxin gene (PVL, tst, sem, seg)</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1</td>
<td>K, ER, SXT</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>L</td>
<td>1</td>
<td>Multiresistant</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

K- kanamycin, ER-erythromycin, SXT-cotrimoxazole; multiresistance- resistance to more than three antibiotic groups.

FIGURE 1: PFGE showing large genomic DNA fragments after digestion with restriction enzymes (Standard controls and test strains). Since the bacterial chromosome is typically a circular molecule, this digestion yields several linear molecules of DNA. After digestion of the DNA and electrophoresis through an agarose gel, if the DNA banding pattern between any two isolates is identical, then these isolates are considered the same strain. Conversely, if two isolates are not the same strain, then the sites at which the restriction enzymes act on the DNA and the length between these sites would be different; thus their DNA banding patterns will be different.

DISCUSSION

Antimicrobial resistance has been noticed as one of the paramount microbial threats in the twenty-first century (20). *S. aureus* has always been a stumbling block for antimicrobial chemotherapy and the introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of this pathogen (21). Surveillance on the antimicrobial susceptibility patterns of *S. aureus* is important in understanding new and emerging resistance trends and in the management of both hospital and community-acquired infections. Methicillin resistance of *S. aureus* remains to be a significant problem. Rapid and accurate determination of methicillin resistance is important for initiation of appropriate antimicrobial therapy. Misdiagnosing this resistance leads to treatment failures and spread of infections with these resistant strains. The increasing reports about vancomycin resistance among *S. aureus* isolates mandate the use of glycopeptide antibiotics which yet appear to be the only choice, sparingly. Therefore, striving for the identification of methicillin resistance as soon as bacterial growth is observed is the only way to limit the superfluous use of glycopeptide class on sensitive isolates.

A major problem in the treatment of *S. aureus* infections is the multidrug resistance pattern of the pathogen to a number of antibiotics. In the last few years, understanding of the genetic basis for methicillin resistance has advanced significantly. Multi-resistant MRSA have been reported to be relatively high in African countries including Morocco, Kenya and Cameroun (22), but their antibiotypes were not determined. This study has provided baseline information for physicians, clinical microbiologists and public health officials on critical issues regarding pathogen specific therapy. The isolation of MRSA in health institution indicates that adequate steps in limiting spread are urgently needed. Also, for the first time two clones (H and L) according to the PFGE classifications have been identified in Nigeria with one agr type (1) but no detection of any of the toxin genes tested. Continuous surveillance on resistant pattern and molecular characterization or epidemiological typing in order to generate isolate-specific genotypic or phenotypic characters that can be used to elucidate the source and route of spread of MRSA infection in Benin City, Nigeria is relevant and of utmost importance.

ACKNOWLEDGEMENT

The assistance of the Laboratory of Professor Iris Spiliopoulou, Department of Microbiology School of Medicine, University of Patras, Greece is greatly appreciated.
REFERENCES

PCR DETECTION OF ENTAMOEBA HISTOLYTICA IN MICROSCOPICALLY POSITIVE STOOL SAMPLES OF HOSPITAL PATIENTS IN SOROTI, EASTERN UGANDA

Ekou1,3*, J., Nakavuma1J.L., Erume4, J. & M.Ocaido2

1. Department of Veterinary Parasitology and Microbiology, Makerere University
   P.O Box 7062, Kampala, Uganda

2. Department of Wildlife and Animal resources management, Makerere University
   P.O Box 7062, Kampala, Uganda

3. Department of Animal Production and Management, Busitema University
   P.O Box 236, Tororo, Uganda

* Corresponding author: jekou@vetmed.mak.ac.ug

ABSTRACT

Amoebiasis is an infection caused by water borne protozoan parasite Entamoeba histolytica. In Uganda where sanitation infrastructure and health education was not adequate, amoebiasis was thought to be still an important health problem. However there was little or no data on prevalence of this very important protozoan infection. In addition, microscopy remained the main method for the diagnosis of amoebiasis but could not differentiate between Entamoeba dispar /moshkovskii and Entamoeba histolytica infections. This made determination of true prevalence of Entamoeba histolytica infections difficult. It was against this background that this study was designed to carry out species specific diagnosis of Entamoeba histolytica and Entamoeba dispar /moshkovskii in Uganda where these species had been reported to be endemic. This study used microscopy and polymerase chain reaction amplification of Serine-rich Entamoeba histolytica (SREHP) gene. It was shown that 36.7% (n=22) of the samples initially diagnosed as positive by microscopy were positive by PCR. The true prevalence of E. histolytica and E.dispar /moshkovskii was found to be 7.31% and 12.6% respectively. It was concluded that Entamoeba infection in Soroti, Eastern Uganda is more frequently due to E. dispar /moshkovskii (13.3%) the non-pathogenic forms than to E. histolytica, the pathogen (7.31%).

Key words: Entamoeba histolytica, Microscopy, Polymerase chain reaction, Prevalence.

INTRODUCTION

New efforts are being made to improve the understanding of the epidemiology of the helminths and intensifying the control efforts against these parasites. In contrast, relatively few studies are being carried out in this direction for the intestinal protozoa (1). Microscopy remains the main method used for the diagnosis of amoebiasis in most African countries, however, it cannot differentiate between Entamoeba dispar and Entamoeba histolytica. In Bangladesh, only 40% of patients diagnosed by microscopy were proven to have E. histolytica infection when specific methods were used. In addition, the accuracy of this method in detecting Entamoeba histolytica depends highly on skills of the technician and has been shown to be less sensitive and less specific as compared to other methods such as immuno-florescence (IFA), antigen detection and PCR(2).

Molecular methods, such as PCR, have aided in alleviating some of the sensitivity and specificity
deficiencies associated with traditional methods for the detection of protozoan pathogens. A number of PCR-based assays like gene amplification with specific primers, multiplex PCR, restriction fragment length polymorphism and real-time PCR have been developed for the identification of *E. histolytica* infections (3). In Mexico, *E. histolytica* prevalence of 25.3% in the HIV/AIDS group and 18.5% in the HIV-negative group was described using PCR (4). Dhawan (5) estimated the prevalence rate of amoebiasis in the United States to be approximately 4% with *E. dispar* infection, which is always asymptomatic, being 10 times more common than *E. histolytica* infection. Despite the development of sensitive antigen-based and molecular techniques, there was no information on the true prevalence of *E. histolytica* in Uganda (6).

Very few studies in Africa have used molecular methods to determine the prevalence of *E. histolytica*. As a consequence, large gaps remain on prevalence rates of *Entamoeba histolytica*. Estimates of prevalence rates of *E. histolytica* is an important decision making tool in allocation of limited public health resources, its treatment, prevention and control. Also feasibility of developing an amoebiasis vaccine depends on the estimation of the disease burden among the populations in the high risk areas (7, 8). It was against this background that this study was designed to carry out species specific diagnosis of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* in Uganda where *Entamoeba histolytica* and *Entamoeba dispar* had been reported to be endemic (9).

**MATERIALS AND METHODS**

*Isolation of amoebic DNA from isolates of Entamoeba histolytica*

Sixty faecal samples initially diagnosed as positive by microscopy were stored frozen. For DNA isolation, 200 µl of faecal suspension (0.5 g/ml PBS) was added to 200 µl of 4% polyvinylpolypyrrolidone (PVPP) (Sigma) suspension and heated for 10 min at 100 °C (10). DNA isolation was then done according to the method of Samie et al. (11). The genomic DNA was purified from stool samples using the QiAamp DNA Stool Mini Kit from Qiagen (Qiagen GmbH, Hilden, Germany) with some modifications. Briefly, 250 µl of liquid stool or diluted stool material was added to 50 µl of potassium hydroxide and 15 µl of 1 mol/L dithiothreitol and mixed thoroughly. After a 30-minute incubation period at 65°C, 8.2 µl of 25% HCl and 80 µl of 2 mol/L Tris-HCl (pH 8.3) were added to the mixture. After a brief vortexing, the protocol was continued with the Qiagen mini kit following the manufacturer’s instructions.

**PCR amplification of Ser-rich Entamoeba histolytica Protein gene**

The amoebic *Ser-rich* protein repeats were amplified using PCR and primers specific for *E. histolytica*. An *E. histolytica* *Ser-rich* protein-specific sense primer was GCTAGTCCTGAAAAGCTTGAAGAAGCTG (Primer1), while an *E. histolytica* *Ser-rich* protein-specific antisense primer was GGACTTGATGCAGCATCAAGGT (12). The procedure was as follows: all solutions were gently vortexed and briefly centrifuged after thawing. Using a thin-walled PCR tube on ice, the reaction mixtures were added as follows: 10 X PCR buffer 2.5 µl, 10 mM dNTPs 5.0 µl, 25 mM MgCl₂ 5.0 µl, 50 pmol Primer1 1.0 µl, 50 pmol Primer2 1.0 µl, Taq polymerase 0.3 µl, distilled water 1.5 µl, DNA sample 3.0 µl. Samples were then placed in a thermocycler and set as follows: denaturation at 94°C for 3 minutes, annealing at 55°C for 1 minute, extension at 72°C for 2 minutes and whole process repeated for 35 cycles. The PCR products were identified on 12% polyacrylamide gel electrophoresis at 80 volts for four hours. Standard strain *E. histolytica* HM-1: IMSS to be used as positive control, could not be obtained. However, a negative control was used. The negative control was prepared as follows: 10 X PCR buffer 2.5 µl, 10 mM dNTPs 5.0 µl, 25 mM MgCl₂ 5.0 µl, 50 pmol Primer1 1.0 µl, 50 pmol Primer2 1.0 µl, Taq polymerase 0.3 µl, distilled water 4.5 µl.

**RESULTS**

It was shown that only 22 of the 60 samples (33.3%) initially diagnosed as positive by microscopy were positive by PCR (Figure 1). Two strains of *E. histolytica* were common, being detected in five separate patients. The patients whose samples were identified as: 4, 9, 11, 12, 16 seemed to be infected by a similar strain. While patients whose sample identity were: 2, 7, 18, 20 and 22 also seemed to be infected with another strain. However, each of the six strains (8, 10, 13, 14, 15, 21) were detected in only one patient.
DISCUSSION

*Entamoeba histolytica* infections are common in Sub-Saharan Africa but the true prevalence of infection and disease caused by *Entamoeba histolytica* was unknown for Uganda and most of Sub-Saharan Africa. This was attributed to the fact that until relatively recently the laboratory differentiation of *E. histolytica* from the morphologically identical non-pathogenic amoebic species *E. dispar/moshkovskii* was not possible. However, differential identification of *E. histolytica* and *E. dispar* is essential for both appropriate patient treatment and epidemiological purposes. Despite the development of sensitive antigen-based and molecular techniques; there was no other information on the diversity of *E. histolytica* strains in Uganda. As a consequence, large gaps remained in our knowledge of species prevalence rates. To address this, species specific diagnosis of *E. histolytica* was performed among isolates from Soroti.

In this study, the amoebic Ser-rich protein gene repeats were amplified using direct PCR using primers that are specific for *E. histolytica*. The results showed that only 33.3% of samples initially diagnosed as positive by microscopy were positive by PCR (Figure 1). This gave overall true prevalence 7.31% of *E. histolytica*.

These results are consistent with earlier observations that *Entamoeba* infection in Africa is more frequently due to *E. dispar* than to *E. histolytica*. Similar observations have been made in Brazil, Nicaragua, and Italy (13). Australia exhibits the highest frequency of *E. dispar* (73.3%) and *E. moshkovskii* (60.7%) infection, which were detected in a population referred to as a clinical laboratory service where 2.9% of samples were microscopically positive for *Entamoeba* cysts (13). In contrast, in countries along the Pacific coast, the frequency and prevalence data

Figure 1: Gel photographs of SREHP PCR products amplified from DNA of *Entamoeba histolytica* positive stool samples from Soroti. 1-22 are the positive samples; M is a 100 bp marker while N is negative control.
for *E. histolytica* infection seem to be higher or similar to those obtained for *E. dispar*. The data available for the frequency of *E. histolytica* infection in the Middle East show that cases in the Gaza Strip in Palestine detected in hospitalized patients exhibited a 69.5% frequency, as compared to Saudi Arabia with a frequency of 2.7% in a similar population (14, 15).

The PCR resulted into mostly single but also multiple bands. This PCR product length polymorphism was thought to result from size variations within the SREHP gene. There is evidence to suggest that the *E. histolytica* SREHP genome is tetraploid (16, 17). This could have accounted for the multiple bands that were observed and may reflect polymorphism among homologous loci on allelic chromosomes. Another factor that could account for multiple bands was the existence of the repeat loci at multiple locations in the *E. histolytica* genome each with a characteristic PCR product. To further support this argument, it has previously been shown that SREHP appears to be a single copy gene only when analyzed using Southern blots. However, when the repeat region is amplified from a clonal line of an *E. histolytica* isolate, often two or three bands are observed, not always of equal intensity but usually quite close in size (18). This suggested that multiple alleles can be present and that the ploidy is likely to be four. The major way in which the alleles differ was said to be in the number of 8 and 12 amino acid repeats that are present, but there are sequence differences between the DNA repeats also. It could also be argued that since there are different strains of *Entamoeba histolytica* in the environment and that humans are continuously exposed to them in a similar manner, it is possible for a single host to be infected by more than one strain at the same time. Multiple bands could then result from infection of a single patient with several different strains of *Entamoeba histolytica*. This can be from the same point source or different sources infected at the same time or different times. Besides parasite mutations during infection can produce modified strains capable of being distinguished from the original strain and thus resulting in multiple bands in the same patient.

In conclusion, Entamoeba infection in Soroti, Eastern Uganda is more frequently due to *E. dispar/moshkovskii* (13.3%) the non-pathogenic forms than to *E. histolytica*, the pathogen (7.31%).

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OCCURRENCE OF METHICILLIN AND VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN UNIVERSITY OF ABUJA TEACHING HOSPITAL, ABUJA, NIGERIA.

Akanbi B. O. & Mbe J. U.

1Department of Biological Sciences, PMB 117, University of Abuja.

ABSTRACT

The susceptibility of clinical isolates of *Staphylococcus aureus* from a hospital to seven antibiotics; namely ofloxacin, vancomycin, oxacillin, erythromycin, ampicillin and gentamicin was examined. The isolates were recovered from wound, skin, urine, blood, vaginal, cerebrospinal fluid and ear infections. After confirmation as *S. aureus* through gram stain and biochemical tests, the antibiogram of each isolate was determined using the disk diffusion method. A total of 214 *S. aureus* isolates were examined of which 28 (13.1%) were resistant to methicillin. Of these 25% were sensitive to ofloxacin, 85.7% to vancomycin, 10.7% to erythromycin 0% to ampicillin and gentamicin. Four (14.3%) of the Methicillin resistant isolates were also resistant to vancomycin and all other antibiotics. There was a significant difference in the sensitivity pattern between inpatient isolates and outpatient isolates in this study (p < 0.05). This study established the presence of methicillin resistant *Staphylococcus aureus* (MRSA) as well as VRSA in this locality and hence the need to implement measures that will limit the dissemination of these strains in the hospital and the community.

INTRODUCTION

*Staphylococcus aureus* is a gram-positive pathogen that causes a wide range of infections including life-threatening ones. *S. aureus* causes bloodstream infections, skin and soft tissue infections as well as pneumonia [1]. Rates of *S. aureus* infection have increased during the past 2 decades [2]. Bacteremia due to *S. aureus* has been reported to be associated with very high mortality rates [3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has occurred in many countries since its discovery in 1961 [4]. The emergence of antibiotic-resistant strains, particularly MRSA is recognized as very serious health problem because of difficulties in combating these strains [5].

Infections with antibiotic-resistant organisms generally result in higher morbidity and mortality rates than are similar infections with antibiotic-susceptible strains [6]. In recent years, clinicians have been concerned by the increased frequency of MRSA infections [7]. This resurging MRSA problem seems to be based on the lack of potent therapeutic agents having an unequivocal cell-killing effect, and thus capable of eliminating MRSA from the patient's body [8]. MRSA strains are often resistant to multiple antibiotics and pose serious challenges in both hospitals and the community [9]. MRSA infections are associated with significant increases in mortality, longer hospital stays and higher inpatient costs compared to patients with methicillin susceptible *S. aureus* [10,11]. Initially MRSA was considered a nosocomial problem, however community-associated methicillin-resistant *S. aureus* (CMRSA) has become the most frequent cause of skin and soft tissue infections [12].

Since the emergence of MRSA, vancomycin has been the most reliable therapeutic agent against infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). However, in 1997, the first clinical isolate of *Staphylococcus aureus* with reduced susceptibility to vancomycin was reported, [13] and by 2001 Vancomycin resistant *S. aureus* (VRSA) strains had been isolated in USA, France, Korea, South Africa, and Brazil [8]. A certain group of *S. aureus*, designated hetero-VRSA, frequently generate VRSA upon exposure to vancomycin, and are associated with infections that are potentially refractory to vancomycin therapy [8]. The present study sought to determine the prevalence of MRSA and VRSA and hence the extent of the problem in clinical isolates of *S. aureus* since there is a paucity of published data in this locality.

MATERIALS AND METHODS

*S. aureus* isolates were obtained from various clinical samples at the Microbiology Laboratory University of Abuja Teaching Hospital and National Hospital from April 2010 to June 2011. For each specimen of a patient only one positive isolate was included in the study. Specimens were categorized as inpatients or outpatients using the definition of nosocomial infection as a localized or systemic condition that results from adverse reaction to the presence of an infectious agent not present or incubating at the time of admission to the hospital according to the Centers for Disease Control and Prevention [14]. *S. aureus* strains were identified by colony morphology, pigmentation and growth on Mannitol salt agar (Merck), Gram stain, catalase activity, and slide coagulase tests. Using a 0.5 McFarland-equivalent suspension of organisms inoculated on a Mueller-Hinton agar plate (Oxoid, Basingstoke), Isolates were tested using antibiotic discs obtained from Oxoid, Basingstoke. The isolates were tested for methicillin resistance using oxacillin discs (1 µg) and for vancomycin resistance using vancomycin discs (5 µg) as recommended by Andrews [15]. Susceptibility of the isolates to other antibiotics namely; gentamicin (10 µg), Ofloxacin (5 µg), erythromycin (5 µg),
ampicillin (25 µg) was also determined as recommended by Andrews [15].

Statistical Analysis

Chi-square ($\chi^2$) test was performed using The Primer of Biostatistics software (McGraw-Hill version 4.0). It was used to analyze data from resistance of inpatient and outpatient MRSA and MSSA strains to antibiotics to determine if there were significant differences. $P$ values $< 0.05$ were considered significant.

RESULTS

The total number of $S. aureus$ strains examined was 214. Of these 33(15.4%) originated from the wound, 30(14%) from urine, 85(39.7%) from the blood, 10(4.7%) from the vagina, 6(2.8%) from hair scalp, 6(2.8%) from ear infections, 42(19.6%) from the skin and 2(0.9%) from cerebrospinal fluid. The total number of isolates from inpatients was 114 while 100 were from outpatients.

Out of the total number of 214 isolates tested, 28 were MRSA and of these 4 were also VRSA. 20 of the MRSA were from inpatients and 8 MRSA were from outpatients. Vancomycin resistance was not detected in any of the outpatient isolates.

The highest number of MRSA was detected in blood isolates (11) followed by wound (7), 4 were detected in urine and skin isolates whilst 2 were detected in cerebrospinal fluid. Generally the number of MRSA detected in inpatients was higher than in outpatients. In terms of proportion of number of MRSA detected to the total number of isolates examined for each type of specimen, cerebrospinal fluid specimens had the highest value with all two isolates being MRSA i.e. 100%. This was followed by wound swabs in which 7 out of 33 (21%) were MRSA, urine samples with 4 out 30 (13.3%), blood with 11 out of 85 (12.9%) and skin swabs 4 out 42(9.5%) respectively. MRSA was not detected from vaginal swab, hair scalp and ear infections samples. As earlier stated out of the 28 MRSA isolates, 4 were also VRSA. These originated from cerebrospinal fluid, wound and blood samples. One out of the 4 MRSA isolated from wound was also a VRSA, 1 out of 2 from cerebrospinal fluid was VRSA while 2 out of 10 of blood were MRSA.

Apart from oxacillin and vancomycin other antibiotics tested against the isolates were erythromycin, gentamicin, ampicillin and ofloxacin.

### TABLE 1: DISTRIBUTION OF MRSA, MSSA AND VRSA STRAINS ACCORDING TO THEIR ORIGIN.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>MRSA (VRSA)</th>
<th>MSSA(VRSA)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inpatient</td>
<td>Outpatient</td>
<td>Inpatient</td>
</tr>
<tr>
<td>Wound</td>
<td>4(1)</td>
<td>3(0)</td>
<td>14(0)</td>
</tr>
<tr>
<td>Urine</td>
<td>2(0)</td>
<td>2(0)</td>
<td>16(0)</td>
</tr>
<tr>
<td>Blood</td>
<td>10(2)</td>
<td>1(0)</td>
<td>50(0)</td>
</tr>
<tr>
<td>Vaginal</td>
<td>0(0)</td>
<td>0(0)</td>
<td>6(0)</td>
</tr>
<tr>
<td>Hair scalp</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Ear infections</td>
<td>0(0)</td>
<td>0(0)</td>
<td>2(0)</td>
</tr>
<tr>
<td>Skin</td>
<td>2(0)</td>
<td>2(0)</td>
<td>6(0)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>2(1)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

Values in brackets indicate the number of VRSA in a particular group out of the total.

### TABLE 2 ANTIBIOTIC RESISTANCE PATTERNS IN MRSA AND MSSA ISOLATES TO SELECTED ANTIBIOTICS

<table>
<thead>
<tr>
<th>ANTIBIOTIC</th>
<th>MRSA</th>
<th>MSSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inpatient Isolates</td>
<td>Outpatient Isolates</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td></td>
<td>Inpatient isolated</td>
<td>Outpatient isolated</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>
Approximately fourteen percent of MRSA isolates were also resistant to vancomycin, 75% to ofloxacin, 89.3% to erythromycin and 100% to ampicillin and gentamicin respectively. Among the MSSA none (0%) was resistant to vancomycin, 19.4% was resistant to ofloxacin, 25.3% to erythromycin, 59.6% to gentamicin and 98.9% to ampicillin respectively. The difference in resistance of inpatient and outpatient MRSA strains to other antibiotics was significantly different (p <0.05). Similarly, the difference in resistance of inpatient and outpatient MSSA strains to other antibiotics was also significantly different (p <0.05).

In all, three of VRSA were not susceptible to any of the other antibiotics used in the study while one was susceptible to ofloxacin.

DISCUSSION

MRSA has caused problems in most hospitals worldwide and increasing numbers have been reported in a number of countries. There have been significant increases in methicillin resistance in clinical strains of \textit{S. aureus} isolates between 1999 and 2002 in European countries, particularly Belgium, Germany, Ireland, the Netherlands and the United Kingdom [16]. MRSA prevalence varied widely, from less than 1% in northern Europe to greater than 40% in Southern and Western Europe [16].

In this study 13.1% of \textit{S. aureus} isolates were resistant to methicillin. This figure is less than 20.6% and 47.8% reported from Southwestern Nigeria [17,18] and 69% reported in Zaria northern Nigeria [19]. The low rate of MRSA in this study may be due to low level of abuse of antibiotics in this locality by both health practitioners and in the community since emergence of resistant strains has been largely due to antibiotic abuse. Moreover MSSA strains were largely susceptible to other antibiotics and none was resistant to vancomycin.

Most of the MRSA isolates were also resistant to other antibiotics. The presence of \textit{mec} A gene complex which specifies the production of an abnormal penicillin binding protein PBPs that has a decreased affinity for binding \beta-lactam antibiotics results in resistance to methicillin and also to all \beta-lactams including penicillins and cephalosporins also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. Thus, cross-resistance to non-\beta-lactam antibiotics such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin is common [7].

MRSA was higher in inpatients expectedly because of established MRSA risk factors, such as recent hospitalization, surgery, residence in a long-term care facility, receipt of dialysis, or presence of invasive medical devices [20]. Detection of MRSA from outpatients warrants more investigation because community associated MRSA (CA-MRSA) has emerged in the community with clinical, epidemiologic, and bacteriologic characteristics distinct from healthcare-associated MRSA [21,22,23]. CA-MRSA isolates also tend to be resistant to fewer antimicrobial classes, possess different toxin genes, and carry a different type of the gene complex known as staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec}), which contains the \textit{mec} A methicillin-resistance gene [24, 25]. The resistance of outpatient isolates to different antibiotics appeared to be less than those from inpatients but this remains to be confirmed by larger studies because of the limited data from the present study. The higher susceptibility pattern of MSSA to antibiotics seems to confirm the tendency of MRSA to acquire resistance genes to other antibiotics.

Vancomycin resistance was observed in 4 isolates and only one of which was susceptible to any other antibiotics. The reported prevalence rate of VRSA in southern parts of Nigeria range from 0% to 6% among clinical isolates in agreement with the present study [17,18]. A prevalence rate 57.7% has also been reported in Zaria northern Nigeria [26] and in another study in non clinical isolates a prevalence rate 89% was reported [19].

The difference in rates of vancomycin resistance is probably reflects differences in levels of over prescription and abuse in different parts of the country. This study as well as others might however have underestimated vancomycin resistance because of the inability of routine antimicrobial susceptibility test methods to detect heterogeneous vancomycin-intermediate \textit{Staphylococcus aureus} (hVISA) and vancomycin-intermediate \textit{Staphylococcus aureus} VISA detected in different continents [27-30]. Vancomycin resistant strains are a source of concern because until recently, vancomycin was the only uniformly effective treatment for staphylococcal infections particularly MRSA. Resistance to vancomycin severely limits treatment options as seen in this study.

Although the incidence of MRSA and VRSA observed in this study has not reached epidemic proportions, it is still very important to put in measures to contain their spread and continue surveillance both in healthcare facilities and in the community. The spread of these pathogens in the community can result in skin infections and, less commonly, invasive infections among otherwise healthy adults and children in the community.

REFERENCES


ANTIBACTERIAL ACTIVITIES OF DIFFERENT BRANDS OF DEODORANTS MARKETED IN OWERRRI, IMO STATE, NIGERIA

EGBUOBI, R. C., OJIEGBE, G. C., DIKE-NDUDIM, J.N. & ENWURU, P. C.

1, DEPARTMENT OF MEDICAL LABORATORY SCIENCE, IMO STATE UNIVERSITY, OWERRI. 2, ST JOHNS MEDICAL LABORATORY, OWERRI.

CORRESPONDENCE: EGBUOBI R. C. P. O. BOX 3739, OWERRI E-mail address: neekyrichy@yahoo.com.

ABSTRACT

Body odour caused by bacterial growth is of great concern to those affected. To ameliorate this, deodorants, which are substances applied to avert body odour, are designed and used. These deodorants are claimed to have antibacterial activities. This work was therefore, carried out to determine the antibacterial activities of the deodorants marketed in Owerri municipality. Owerri is the capital of Imo State of Nigeria. To determine this, twenty (20) deodorants from ten (10) different manufacturers made up of fourteen (14) roll-on and six (6) sprays were assessed using punch-hole plate diffusion technique on nutrient agar plates. The deodorants were purchased from Ekeonunwa market, New market, Relief market, and some supper markets, all in Owerri Municipality. These deodorants were tested against Laboratory isolates of Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis obtained from Federal Medical Centre (FMC) and Modesty Medical Laboratory Owerri and reconfirmed using morphological and biochemical tests. Out of the twenty (20) deodorants tested, 18 (90%) were active against the organisms, 17 (85%) were active against Staphylococcus epidermidis, 15 (75%) against P. mirabilis, 11 (55%) against E. coli and 10 (50%) against P. aeruginosa. It was also observed that deodorants “roll-on” were more active 14 (100%) than the deodorants “sprays” 4 (66.6%). From this study, it was observed that the deodorants tested had antibacterial activities.

Key words: Deodorants, Antibacterial, body, odor
INTRODUCTION

Deodorants are substances applied to the body to affect body odour caused by bacteria growth and the smell associated with bacterial breakdown of perspiration in armpits, feet and other body areas. A sub group of deodorants, 'antiperspirants', affect odour as well as prevent sweating by affecting sweat glands. Antiperspirants are typically applied to the underarms, while deodorants may also be applied on the feet and other areas in the form of body sprays.

Human perspiration is largely odourless until it is fermented by bacteria that thrives in hot, humid environments. The human underarm is among the most consistently warm areas on the surface of the human body, and sweat glands provide moisture, which when excreted, has a vital cooling effect. When adult armpits are washed with alkaline P soap, the skin loses its acid mantle, raising the skin environment and disrupting the skin barrier. Many bacteria thrive in this elevated environment. These bacteria feed on the sweat from the apocrine glands and on dead skin and hair cells, releasing hexonic acid in their waste which is the primary cause of body odour. Underarm hair wicks the moisture away from the skin and aids in keeping the skin dry enough to prevent or diminish bacterial colonization. The hair is less susceptible to bacterial growth and therefore is ideal for preventing the bacterial odour. While deodorants, reduce body odour by killing the odour causing bacteria, they do not affect the amount of perspiration the body produces. Antiperspirants, on the other hand inhibit the activity of sweat glands, so less moisture is produced in addition to avoiding unpleasant wetness. These products also decrease odour because there are less sweat for the bacteria to act upon.

The terms ‘deodorants’ and “antiperspirants” are often used interchangeably, although they describe two different products. While the most frequent active ingredient in commercial antiperspirants are Aluminium chloride and Aluminium zirconium tetrachlorohydrate gly, deodorants are usually alcohol-based, containing some antimicrobial agents such as Fernesole, triclosan, Paraben, Zinc riconoleate, triethyl citrate, chlorophyllin copper complex and other metal, chalent compounds that slow bacterial growth. Deodorants exhibit their antimicrobial activities, by reducing the body which in turn inhibits the growth of microorganisms that produce malodour. Another difference between deodorants and antiperspirants is that, while deodorants are considered to be cosmetics products because they only control odour, antiperspirants are actually drugs because they affect the physiology of the body. The first ever deodorant was produced by zingal in the 9th Century in Al-Andalus. Ever since then modernizations have been going on, with some criticisms from the US food and Drug Administration (FDA) and Environmental protections Agency (EPA) which brought about what we now have in the market as present day deodorants.

Deodorants and antiperspirants come in many forms. What is commonly used, varies from country to country. In Europe, aerosol sprays are popular, as are cream and roll-on forms. In the United State, solid or gel forms are dominant. And in Nigeria (especially Owerri), both the sprays and the roll-on brands are common. Many people use and believe in the antibacterial effectiveness of these deodorants, which they hope will prevent or reduce the body odour.

This work therefore, was carried out to determine the antibacterial effect of these deodorants marketed in Owerri, the capital of Imo State of Nigeria.

It is also aimed at comparing the level/degree of efficacy between the two brands marketed in Owerri.

MATERIALS AND METHODS

ORGANISM

The organisms used were laboratory isolates such as Staphylococcus epidermidis, skin normal flora, Proteus mirabilis, Pseudomonas aeruginosa and Escherichia coli which were obtained from Federal Medical Center Owerri (FMC) and Modesty Medical Laboratory Owerri in Imo State of Nigeria from June – November 2011. The organisms were tested, using standard discs and were found to have zones of inhibition of more than 15 mm diameter against pencillin, ampicillin, gentamycin and ampicillin respectively.

These isolates were reconfirmed before being used in this research work. The reconfirmation was done in Modesty Medical Laboratory following the conventional method of identification such as Morphological, Grams staining reaction technique, Motility and Biochemical methods.

PREPARATION OF DEODORANT

A total of twenty (20) deodorants from ten (10) different manufacturers were tested. They were made up of fourteen roll-ons (R1-R14) and six sprays (S1-S6). The deodorants were selected randomly. The fluid of each deodorant roll-on, was aspirated aseptically into sterile tubes.

While that of the spray was sprayed into a sterile container before been used for this work.

SUSCEPTIBILITY TESTING

Plate diffusion technique using the punch-hole method was adopted, oxoid nutrient agar medium which was used was prepared and sterilized according to the manufacturer’s instruction. The nutrient agar plates were inoculated with the bacteria, each plate for each bacterium but was done in duplicate.

The plates were inoculated with the organism after dilution to give about 10^6 cells/ml. Punch holes were made using sterile cock borer of 10mm diameter. Each hole was labelled against the deodorant and filled, allowed to stay for about 30minutes before incubation at 37°C for 18hours. Zones of inhibition were measured using mm ruler. Any deodorant giving an inhibition zone of more than 2mm on either side of the hole was regarded as having antibacterial activity.

RESULTS

The antibacterial activities of twenty (20) deodorants from ten (10) different manufacturers consisting of fourteen (14) roll-ons and six (6) sprays were assessed using agar diffusion
punch - hole technique against *Staphylococcus epidermidis*, (skin normal flora), *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Diameter of the zones of inhibition was measured. Zones less than 2mm on either sides of the hole were considered resistant.

Out of the twenty (20) deodorants that were tested against the organisms, 18 (90%) have zones of inhibition greater than 2mm on each side. Seventeen 17 (85%) showed antibacterial activity against *S. epidermidis*, fifteen 15 (75%) inhibited *P. mirabilis*. Eleven 11 (55%) was active against *E. coli* and ten 10 (50%) inhibited *P. aeruginosa*.

All the deodorants that were active against *S. epidermidis* showed zones of inhibition above 5mm, except R₄, R₁₄, S₁ and S₂ that had 2mm zones of inhibition each and R₁₈ showed the highest zone of inhibition (15mm) against *S. epidermidis*. *P. aeruginosa* was the least sensitive of all the organisms to the deodorants, 10 (50%). Out of the ten deodorants that were active against it, R₈ and R₁₀ showed the highest zone of inhibition 13mm each.

Among the fourteen (14) roll-on deodorants assessed, eleven 11 (78.6%) showed zones of inhibition against all the organisms, fourteen 14 (100%) were active against *S. epidermidis*, ten 10 (71.4%) against *P. aeruginosa*, Eleven 11 (78.6%) against *E. coli* and fourteen 14 (100%) against *P. mirabilis*. Comparing the six (6) spray deodorants assessed, none (0%) was active against all the organisms, but 3 (50%) were active against *S. Epidermidis*, two 2 (33.3%) against *P. mirabilis*. None (0%) was active against *P. aeruginosa* and *E. coli*.

**DISCUSSION**

This work aimed primarily to determine the antibacterial activities of different brands of deodorants marketed in Owerri metropolis of Imo State, Nigeria, as well as comparing the level/degree of efficacy between the two brands marketed in Owerri.

The deodorants roll-on, R₁₁, R₈, and R₁₀ were discovered to have higher level of activity to all the four tested organisms with [(15,8,10,15), (14,13,15,14), and (13,13,13,12)] mm diameter respectively. This peculiar high levels of zones of inhibition reveals the antibacterial efficacies of their various active ingredients (such as triclosan, fanesole, paraben, triethyl citrate and so on). Such level of antimicrobial activity is desirable in the production of deodorants³⁶, and possession of acceptable level of antimicrobial activity by super critical Hops extract have been used as natural deodorants³⁶.

R₄, R₁₃, and R₁₄ on the other hand showed no zone of inhibition to some of the organisms [(2,0,0,4) (9,0,0,6) and (2,0,0,2)] mm diameter respectively. This could mean that their various active ingredients are contained in low concentrations. This is proven by the statement that some bacterial species can develop low level resistance to triclosan at its low bacteriostatic concentrations due to Fable mutations which results in a decrease to triclosan’s effect on ENR-NAD + binding³⁷,³⁸.

Considering sprays, only four sprays (S₄, S₅, S₃S₅) showed zones of inhibition to some of the organisms and among the four sprays that showed the zones of inhibition, only S₅ showed appreciable zone of 6mm diameter to two of the organisms, others showed zones less than 3mm diameter each to one of the organisms only. This may be attributed to the fact that their various active ingredients are contained in very low concentration, regardless of the fact that they were listed on the containers of the sprays in their right proportions. This is in agreement with ¹⁹ who stated that ‘Another way for these bacteria to gain low-level resistance to triclosan is to over express fable because some bacteria have innate resistance to triclosan at low bacteriostatic level.

Again, the proportion of the ingredients may be in their right order, but, because they are in their gaseous states, they do not have long time of contact with the organisms before they evaporate, thereby bringing the concentration in contact with the organism lower than what it should be. This is also in agreement with ³⁹.

Then, looking at the susceptibilities of the test organisms used, the high level of susceptibility shown by *Staphylococcus epidermidis* (85%) could be related to the fact the organism is a normal flora of the skin which is the primary target of deodorants production. *Staphylococcus* along side with *Corynebacterium* and *Propionibacterium* were listed as the notable bacterial skin normal flora³⁰.

*Pseudomonas aeruginosa* showed the lowest susceptibility (50%) to all the deodorants tested. Some of deodorants tested were found to have (0%) activeness against it. This could be because *P. aeruginosa* posses multi-drug efflux that pumps triclosan out of the cells²¹,²².

**CONCLUSION AND RECOMMENDATIONS**

In conclusion, we confirm that Deodorants in Owerri market are active against bacterial growths associated with breakdown of perspiration in armpits, feet, and other areas of the body to produce body odour.

We therefore recommend the use of deodorants to control the body odour caused by bacterial breakdown of lipid and protein in sweat, especially during the Adolescent and adult stages of life, when the apocrine gland is developed and produces sweat rich in protein and fatty acid.

We also recommend to the manufacturers, that enough active ingredients be added in the production of different brands of deodorants to enable them achieve their primary aim of production.

Finally we recommend that the National Agency for Food and Drug Administration and Control (NAFDAC) and the Consumers Protection Council (CPC) should rise up to their responsibility of ensuring that the ingredient listed on the containers of these deodorants and their concentrations are actually corresponding to their real contents to avoid deceiving the poor masses. Also further monitoring researches be done on this aspect.
Fig 1: Shows each deodorant and number of organisms affected.

**Key:** R1,..., R14 = Roll on brands of deodorants (1-14); S1,..., S6 = spray brand of deodorants (1-6)

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12. Time magazine. Corporations: Scalping the Competition July12


UNUSUALLY HIGH PREVALENCE OF ASYMPTOMATIC BACTERIURIA AMONG MALE UNIVERSITY STUDENTS ON REDEMPTION CAMP, OGUN STATE, NIGERIA

Ayoade, F., Osho, A., Fayemi, S.O., Oyejide, N.E. & Ibikunle A.A.

1. Department of Biological Sciences, College of Natural Sciences, Redeemer’s University P.O. Box 812, Redemption Camp Post Office, KM 46, Lagos-Ibadan Expressway, Redemption Camp, Ogun State, Nigeria
2. Correspondence: Dr. Femi Ayoade
E-mail: fayoade22@gmail.com

ABSTRACT

Differences are known to occur in prevalence rates in urinary tract infections (UTI) between men and women due to the difference between the urinary tracts of the sexes. Moreover, different organisms are known to infect and cause bacteriuria in men. When urine samples from 55 apparently healthy male students of Redeemer’s University were examined, nine bacteria species including Micrococcus luteus, Viellonella parvula, Micrococcus varians, Streptococcus downei, Streptococcus pneumonia, Bacillus subtilis, Streptococcus pyrogenes, Staphylococcus saprophyticus, and Enterococcus aquimarinus were isolated from the samples. The two most prevalent organisms reported in this study were Micrococcus luteus (40%) and Micrococcus varians (27.3%). The implication of the high prevalence rates (54.5%) of asymptomatic bacteriuria obtained in this population is discussed.

Key words: Asymptomatic UTI, bacteriuria, Micrococcus luteus

INTRODUCTION

Urinary tract infections (UTIs) are caused by the presence and growth of micro-organisms anywhere in the urinary tract and are perhaps the single commonest bacterial infection of mankind (1, 2). Urinary tract infection occurs when bacteria is introduced into the urinary system usually through the urethra, when it gets into the urinary system it multiplies and travels up the urinary tract causing inflammation and irritation along the way. The high incidence of the Escherichia coli in UTIs could be attributed to the fact that it is a commensal of the bowel and that infection is mostly by faecal contamination due to poor hygiene. Other organisms typically reported to be responsible for urinary tract infections include Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella aerogenes. Enterobacter spp., and Proteus spp. While this list is not exclusive, the prevalence and degree of occurrence of one or two of these organisms over the other are dependent on the environment (3).

The prevalence and incidence of Urinary Tract Infections is typically higher in women than in men, which is likely the result of several clinical factors including hormonal effects, behavioural pattern and anatomical differences. This is partially due to short and wider female urethra and its proximity to the anus (3). The risk factors identified with high prevalence of UTIs in young adult females include sexual intercourse, spermicide-based contraception, and a history of UTIs (4).

Asymptomatic bacteriuria is reportedly rare in young adult males accounting for typically approximately 5-6% wherever reported (8). Asymptomatic UTIs occur when urinary tract pathogens enter into the bladder without causing apparent symptoms. It is defined by the presence of at least 10^5 colony forming units (CFU) of organism per millilitre of urine specimens in the absence of symptoms of infection referable to the urinary tract (5). Factors that have been reported to predispose young men to urinary tract infections include poor personal hygiene, sexual intercourse, urethritis due to gonorrhoeal infections, homosexuality, and lack of circumcision (6, 7, 8, 9).

Reports from a previous work conducted on the same population of Redeemer’s University students showed a 26.7% prevalence of bacteriuria in males when compared to 73.3% among females with an overall prevalence of 25% in the same population (10). A re-examination of the males in this population was necessitated by the unusually high prevalence reported for males in the previous study. The present study investigates the UTI-causing organisms and their frequency of occurrence in the male population of Redeemer’s University students. Possible explanations for these observations are discussed.

MATERIALS AND METHODS

Mid-stream urine was collected randomly from apparently healthy male undergraduates of Redeemer’s University. Informed consent form written in English was administered to each person
whose urine was collected. The samples were collected into sterile plastic disposable bottles, refrigerated and examined within 2-4 hours of collection. The students were apparently healthy and symptoms free at the time samples were collected. Students on antibiotic treatment within one week of the study were avoided.

55 Urine samples were examined using standard methods (11). A 30 morse gauge, 3.26mm calibrated wire loop capable of delivering 0.001 ml of urine was used for culturing on Cysteine-Lactose Electrolyte Deficient (CLED) and MacConkey agar. The culture plates were incubated aerobically at 37°C for 24 hours. Culture plates without visible growth were further incubated for an additional 24 hours before being discarded. The number and types of colonies grown on the medium (CLED) was recorded as being insignificant when samples gave a colony count of less than $10^4$ CFU ml. Samples with colony count equal to or greater than approximately $10^5$ CFU ml of the urine samples were considered to have significant bacteriuria. Bacterial isolates were identified based on a combination of cultural, morphological and biochemical characteristics (12).

RESULTS

The present results show a 54.5% occurrence of significant bacteriuria among the total of 55 samples examined (Table 1). A total of nine bacteria species were isolated from the urine samples and these were Micrococcus luteus, Viellonella parvula, Micrococcus varians, Streptococcus downei, Streptococcus pneumonia, Bacillus subtilis, Streptococcus pyogenes, Staphylococcus saprophyticus, and Enterococcus aquimarinus. The frequency of occurrence of these species is shown in Table 2. Micrococcus luteus was the most frequently encountered organism at 40% followed by Micrococcus varians with a frequency of occurrence of 27.3% (Table 2).

<table>
<thead>
<tr>
<th>Table 1: Colony Count of Bacteria Found in Urine Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of occurrence</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Samples with significant asymptomatic bacteriuria ($\geq 10^5$)</td>
</tr>
<tr>
<td>Samples with insignificant asymptomatic bacteriuria ($\leq 10^4$)</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Samples with $\geq 10^6$ CFU bacteria colonies per ml of urine samples were considered to have significant bacteriuria.
TABLE 2: FREQUENCY OF OCCURRENCE OF BACTERIA ISOLATES

<table>
<thead>
<tr>
<th>Bacteria isolates</th>
<th>No of samples</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus luteus</td>
<td>22</td>
<td>40.00</td>
</tr>
<tr>
<td>Viellonella parvula</td>
<td>5</td>
<td>9.10</td>
</tr>
<tr>
<td>Micrococcus varians</td>
<td>15</td>
<td>27.27</td>
</tr>
<tr>
<td>Streptococcus downei</td>
<td>3</td>
<td>5.45</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>2</td>
<td>3.64</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>3</td>
<td>5.45</td>
</tr>
<tr>
<td>Steptococcus pyrogenes</td>
<td>1</td>
<td>1.82</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>3</td>
<td>5.45</td>
</tr>
<tr>
<td>Enterococcus aquimarinus</td>
<td>1</td>
<td>1.82</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>55</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

DISCUSSION

The difference in urinary tract infections between men and women arises from the difference between the urinary tracts of the sexes. Moreover, different organisms are known to infect and cause bacteriuria in men and the prevalence is known to vary even among men at different ages (7). The present result shows that the causal organisms isolated from samples in the present study is mostly different from those found in the mixed population comprising male and female students of Redeemer’s University examined in a previous study (10). Only a minimal occurrence of members of the genus Staphylococcus and Streptococcus were found in samples examined in both studies.

The two most prevalent organisms reported in this study Micrococcus luteus and Micrococcus varians are known to be commensal organisms found commonly in skin microflora that may cause opportunistic infections particularly in immunocompromised individuals such as those with conditions such as recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis, and cavitating pneumonia (13). Moreover, M. luteus and M. varians are obligate aerobes found in soil, dust, water and air and as such have been implicated in the infection of the human upper respiratory tract particularly in individuals with poor hygiene that enjoy active living and sports outdoors (13, 14, 15). Since the urine samples examined in the present study were obtained from apparently healthy male students of Redeemer’s University, the high prevalence of asymptomatic bacteriuria obtained in this study may be due predominantly to poor hygiene on the part of the male students who understandably are prone to engage more in active outdoor activities such as sports than their female counterparts and are less disposed to hygienic practices such as frequent bathing, frequent washing of hands, washing of private parts after urinating and so on.
REFERENCES


PREVALENCE AND SUSCEPTIBILITY PATTERNS OF CLINICAL ISOLATES OF ESCHERICHIA COLI TO VARIOUS ANTIMICROBIALS IN A CLINICAL MICROBIOLOGY LABORATORY IN SOUTH-SOUTH NIGERIA

Oreh a,*, N. C., Esimone b, C. O. & Ekwunife c, O. I.

a. Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State; b. Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State; c. Department of Clinical Pharmacy and Pharmacy Management, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State.

Correspondence: Oreh, N. C. E-mail: chineizukanne@yahoo.co.uk

RUNNING TITLE: ANTIMICROBIAL SUSCEPTIBILITY OF ESCHERICHIA COLI

ABSTRACT

The purpose of this study is to determine the prevalence of Escherichia coli as an aetiologic agent in bacterial infections and its antimicrobial susceptibility patterns to ciprofloxacin, ofloxacin, norfloxacin, pefloxacin, gentamycin and cotrimoxazole as a guide for empiric therapy. A retrospective study was carried out using a clinical microbiology laboratory in Nigeria. Data retrieved include number of E. coli isolates, sources of the isolates and their antimicrobial susceptibility to various fluoroquinolones, gentamycin and cotrimoxazole between 2005 and 2009. Statistical analysis was carried out using SPSS version 14, Chicago IL. Out of a total of 906 bacterial isolates, E. coli accounted for 23 % (211) of the isolates. Thirty-eight percent (38.39 %) was isolated from urine samples, 27.96 % from high vaginal swab samples, 24.17 % from stool samples, 0.95% from urethra swabs, 1.9% from wound swabs and 6.6% from semen samples. There was poor level of susceptibility to norfloxacin (2.2%) and cotrimoxazole (23.7%), susceptibility to ofloxacin, ciprofloxacin and pefloxacin were 51.1%, 54.7% and 52.5% respectively, that of gentamycin was 51.8%. The trends across the years showed a significant increase in susceptibility to ciprofloxacin, pefloxacin and ofloxacin in 2007 after which it started reducing, while norfloxacin’s susceptibility was low across the five years with maximum susceptibility at 9.1% in 2006. There was an increase in susceptibility to gentamycin as the susceptibility levels of the fluoroquinolones were reducing. There should be continuous surveillance of antimicrobial susceptibility patterns and empiric treatment with fluoroquinolones discouraged, especially for non urinary tract infections.

KEYWORDS: Antimicrobial susceptibility, Nigeria, Escherichia coli, fluoroquinolones, gentamycin

INTRODUCTION

Antimicrobial resistance is a worldwide problem and it is one of the greatest challenges to health care delivery[1]. There is tremendous variability in antimicrobial resistance patterns not only among pathogens causing various clinical infections but also in different geographical regions and over time [2]. Surveillance of susceptibility patterns of pathogens to antimicrobial agents is therefore, important in the monitoring and detection of increase in resistance [3-5]. It is very helpful for clinicians in prescribing antimicrobial agents especially in cases where empiric treatment is employed. In Nigeria, as is the case in many developing countries, empiric therapy is often employed as a result of inadequate staffing and laboratory facilities [6].

E.coli has been identified as a predominant pathogen for various bacterial infections especially urinary tract infections [7-11]. Fluoroquinolones have been proven to be highly effective broad spectrum agents especially against those infections caused by Gram-negative organisms[12] Due to high resistance to cotrimoxazole, fluoroquinolones became the first drug of choice for empiric treatment of urinary tract infections and other infections caused by Gram-negative bacteria and its widespread use has resulted in the rise in their resistance[13] Despite the regular use of empiric treatment in Nigeria, data on antimicrobial susceptibility patterns of various pathogens are scant. This study was therefore carried out to determine prevalence of E. coli as an aetiologic agent in community acquired bacterial infections and its antimicrobial susceptibility patterns to different fluoroquinolones, as well as gentamycin and cotrimoxazole, as a guide for empiric therapy.
METHODS

This was a single centre retrospective study done between January 2005 and December 2009 in a clinical microbiology laboratory in South-south Nigeria. Data retrieved from microbiology laboratory records included number of E. coli isolates, sources of the isolates and antimicrobial susceptibility to ciprofloxacin, ofloxacin, pefloxacin, and norfloxacin (fluoroquinolones) and also cotrimoxazole and gentamycin, popularly used in the country for the treatment of bacterial infections. Isolates collected from the same specimen source within seven days were excluded. Samples were inoculated on blood agar, MacConkey agar and urine samples on cystine lactose electrolyte deficient (CLED) agar. Culture plates were incubated at 37°C for 24 hours. Identification was done using Gram staining and confirmatory tests were carried out. Antimicrobial susceptibility test was carried out using antibiotic discs on Mueller Hinton agar. Antibiocic disc used were gentamycin (10mcg), cotrimoxazole 25mcg, ciprofloxacin (10mcg), ofloxacin (10mcg), norfloxacin (10mcg) and pefloxacin (10mcg). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Susceptibility tests were classified into two categories – susceptible and resistant, with all intermediate susceptibility classified as resistant. For quality control of susceptibility tests, E. coli ATCC 27922 was used.

Analysis of data was carried out using SPSS version 14, Chicago IL. Frequency of E. coli and susceptibility results were expressed as percentages.

RESULTS

There was a total of 906 bacterial isolates within the time frame (2005-2009) included in this study. Of this 906, E. coli accounted for 23% (211). Frequency of the 211 E. coli isolates from various sources is shown in Fig. 1. Table 2 shows the prevalence of E. coli in the various sources. The total percentage susceptibility of levels of E. coli to the various fluoroquinolones is shown in Table 2.

The susceptibility trends of E. coli to pefloxacin, ciprofloxacin and ofloxacin were similar across the five years (Fig 2). The level of norfloxacin susceptibility across the five years was below 10% with the highest susceptibility level in 2006 at 9.1%. Susceptibility to gentamycin was lowest (27.3%) in 2006 and it kept increasing till it got to its highest (81.8%) in 2009.

### Table 1: Prevalence of Escherichia coli from the various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Prevalence (%)</th>
<th>No of E. coli isolates/total no of samples tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>33.37</td>
<td>81/243</td>
</tr>
<tr>
<td>Stool</td>
<td>26.56</td>
<td>51/192</td>
</tr>
<tr>
<td>HVS</td>
<td>15.86</td>
<td>59/372</td>
</tr>
<tr>
<td>Urethra swab</td>
<td>9.1</td>
<td>2/22</td>
</tr>
<tr>
<td>Wound</td>
<td>15.35</td>
<td>4/26</td>
</tr>
<tr>
<td>Semen</td>
<td>25.93</td>
<td>14/54</td>
</tr>
</tbody>
</table>

### Table 2: Antibiotic susceptibilities of all E. coli isolates to antimicrobials under study between 2005 and 2009

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>% Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFLOXACIN</td>
<td>108/211 (51.1)</td>
</tr>
<tr>
<td>CIPROFLOXACIN</td>
<td>115/211 (54.7)</td>
</tr>
<tr>
<td>NORFLOXACIN</td>
<td>4/211 (2.2)</td>
</tr>
<tr>
<td>PEFLOXACIN</td>
<td>111/211 (52.5)</td>
</tr>
<tr>
<td>COTRIMOXAZOLE</td>
<td>50/211 (23.7)</td>
</tr>
<tr>
<td>GENTAMYCIN</td>
<td>109/211 (51.8)</td>
</tr>
</tbody>
</table>
DISCUSSION

The results show that *E. coli* is an important pathogen in community acquired bacterial infections especially in urinary tract infections, which accounts for 38.39% of all the *E. coli* isolates. Its prevalence in urine samples was also high 33.37%. Prevalence of *E.coli* in stool samples was 26.56% and prevalence in high vaginal swab samples (HVS) was 15.86%. This could be as because of the proximity of the vagina to the anus.

The results also show similar susceptibility levels among the three second generation fluoroquinolones, ciprofloxacin (54.7%), pefloxacin (52.5%) and ofloxacin (51.1%), included in the study. Susceptibility of ciprofloxacin is similar to that found in a study carried out in Tehran [15].

**Fig 1: Frequency of *Escherichia coli* from various sources.**

![Frequency of *Escherichia coli* from various sources](image)

**Fig 2: Susceptibility trends of *E. coli* to various antimicrobials between 2005 and 2009**

![Susceptibility trends of *E. coli* to various antimicrobials between 2005 and 2009](image)
Ciprofloxacin susceptibility was the highest. This is also in line with a study carried out in France [16]. Susceptibility to gentamycin (51.8%) was also similar to that of the fluoroquinolones. However, there was poor susceptibility to cotrimoxazole and norfloxacin. Susceptibility levels for cotrimoxazole in this study was 23.7%. This is similar to other studies carried out in other places [11,17]. Poor susceptibility of E. coli to cotrimoxazole could possibly be as a result of the fact that it has been in use for a long time, hence giving organisms time to develop resistance mechanisms towards it. counter in Nigeria, which is also the case in many other resource poor settings with lax policies, resulting in high and uncontrolled consumption of antibiotics. There has been a steady increase in the level of resistance of commonly used antibiotics. Ciprofloxacin, ofloxacin and pefloxacin were almost at par with susceptibility of just over 50%. In the rural Tamil Nadu study, antimicrobial susceptibility of E. coli from urine isolates to ciprofloxacin was 77.7% [18].

The susceptibility trends across the five years show an increase in susceptibility levels of ciprofloxacin, ofloxacin and pefloxacin. In 2007, susceptibility of ciprofloxacin increased from 35.7% in 2005 to 67.3% in 2007; that of ofloxacin increased from 21.4% in 2005 to 61.8% in 2007 while that of pefloxacin increased from 7.1% in 2005 to 67.3% after which the susceptibility levels started dipping again. This could possibly be due to a reduction in its use prior to 2007 and the subsequent increase in resistance after 2007 might have arisen as a result of increased use. However, this cannot clearly be ascertained from this study. It was also observed that the susceptibility levels of gentamycin, an aminoglycoside, increased (27.3% in 2006 and 81.8% in 2009) as susceptibility levels of the fluoroquinolones were reducing. It should be recommended that antibiotic use be rotated. Antibiotic cycling which is the rotation of antibiotics over a stipulated time period is an important aspect in antibiotic stewardship. A study in Greece [1] showed significant increase in the susceptibility of three important Gram negative pathogens to ciprofloxacin following an eighteen month restriction on the empirical use of fluoroquinolones in the intensive care units.

High level resistance to these fluoroquinolones in this setting might be because of the high level of empiric use of antibiotics as a result of inadequate laboratory facilities, incomplete and wrong usage of antibiotics, high level of abuse as a result of over the counter acquisition of antibiotics and the possibility of having substandard drugs in the market [21]. More studies should be carried out to determine the relationship between level of consumption of these fluoroquinolones and the level of resistance.

Susceptibility of E. coli to norfloxacin was quite low, 2.2% with the highest susceptibility of norfloxacin being 9.1% in 2006. This is contrary to the result obtained from a similar study carried out in rural Tamil Nadu where susceptibility to norfloxacin was 94.44% [18]. According to reports published in 2000, quinolone susceptibility was greater than 95% [12,19]. Generally, the susceptibility levels got in this study is lower than that got from previous studies carried out in developed countries [20]. This could possibly be because of the ease of access to antibiotics over the counter in Nigeria, which is also the case in many other resource poor settings with lax policies, resulting in high and uncontrolled consumption of antibiotics.

There is need for constant antimicrobial surveillance to detect emerging antibiotic resistance patterns. This is also important in the development of hospital antibiograms in order to facilitate the use of the right antibiotics for the treatment of bacterial infections at the local level. Also in Nigeria, where over the counter acquisition of antibiotics have been implicated as one of the reasons for the development of resistance [6], policies should be put in place to control this. This will help curb the scourge of bacterial resistance.

The introduction of electronic laboratory databases which can be analysed with the help of simple software packages such as the WHONET software which can be obtained free from World Health Organisation [22], will greatly help in antibiotic surveillance.

Conclusion

This study has shown that E. coli is a predominant bacterial pathogen in community acquired bacterial infections. There is also a high level of resistance of E. coli to fluoroquinolones in the community. Inasmuch as there is reasonable level of resistance to fluoroquinolones, they will still be useful in the management of urinary tract infections, because they are excreted in the urine unchanged, hence enhancing their effects and E. coli has been indicated as the most common pathogen in urinary tract infections. However, for other sites, it is recommended that fluoroquinolones should not be used for empiric treatment. Proper laboratory tests should be carried out to determine pathogenic organism and sensitivity profile. Furthermore, there is need to set up proper antimicrobial surveillance systems in health institutions through which hospital antibiograms will be developed. Antibiotic cycling should also be implemented to help slow the rate of resistance development. Further studies should be carried out in the region to evaluate the effect of antibiotic utilization on bacterial resistance.

COMPETING INTERESTS: None

AUTHORS’ CONTRIBUTIONS: COE and NCO designed the study protocol, NCO and OIE carried out the analysis and interpretation of data. NCO drafted the manuscript. All authors read and
approved the final manuscript. NCO and OIE are the guarantors of the paper.

ACKNOWLEDGEMENT: The authors wish to appreciate Mr Larry who assisted in data collection.

REFERENCES


CD4 CHANGES IN HAART-NAÏVE HIV POSITIVE PREGNANT WOMEN ON HAART: LOW RESOURCE SETTING EXPERIENCE.

* ADENIJI, A. O. & MUHIBI, M. A.

1. Dept of Obstetrics & Gynaecology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
2. Dept of Haematology, Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria

*Correspondence: Dr ADETUNJI. O. ADENIJI. E-mail: tunji1802@yahoo.com; aoadeniji@lautech.edu.ng Tel: +234-8034305136

ABSTRACT

PURPOSE: PMTCT interventions, especially initiation of Highly active antiretroviral therapy (HAART) has modified the natural history of HIV infection by reducing both peripartal and neonatal HIV infections, but the pattern of the immunologic responses of these pregnant women to HAART remains speculative. It is hoped from this study, to describe the pattern of immunologic response of naïve, HIV positive pregnant women on their first initiation on HAART.

METHODS: This study described the pattern of CD4 changes observed in freshly diagnosed 126 HIV positive pregnant women, stratified into the trimesters of pregnancy and commenced on HAART (Zidovudine, Lamivudine and Nevirapine) for a period of 2 months in pregnancy. CD4 counts were determined at point of recruitment and monthly thereafter using a Partec cytometer counter (Cyflow®). Neonatal outcome was also described.

RESULTS: Our findings suggest better immunologic response and fewer neonatal infections in group of naïve women initiated on HAART while in the first 26 weeks of pregnancy and lesser response in those commenced in the third trimester of pregnancy.

CONCLUSION: Concerted efforts should be directed towards the initiation of HAART prophylaxis before end of 2nd trimester of pregnancy.

Keywords: PMTCT, pregnant women, HAART, CD4 changes

INTRODUCTION

It is well known that the introduction and widespread use of highly active antiretroviral therapy (HAART) has modified the natural history of HIV infection. Significantly, PMTCT practices remain very effective public health interventions in improving maternal health, as well as reducing both peripartal and neonatal HIV infections [1, 2]. While, attempts had been made in general HIV positive patients to characterise both immunologic and virologic responses in patients on HAART in developed countries, such attentions have not been focussed on HIV positive pregnant population, more especially, in our environment with high HIV infection burden. We found no record in our environment of any description of the pattern of immunologic responses of pregnant HIV positive women on antiretroviral drugs. Thus it becomes highly imperative for such, considering the hitherto immunologic changes expected of normal pregnancy. This study thus attempt an assessment of the pattern of immunologic (CD4) changes in naïve HIV positive pregnant women, in the first two months of commencing HAART, with a view to possibly postulate CD4 response rate and recommend the ideal time to initiate HAART in HIV positive pregnant patients.

METHODOLOGY

Patients with double positive parallel rapid test results at EGA 12 - 28 weeks at LAUTECH Teaching Hospital, Osogbo, Nigeria in the year 2008/2009 were prospectively enrolled into the study and stratified into the 3 groups by the trimesters of pregnancy. Institutional Ethical approval was obtained for the study. All patients recruited gave consent and had venous blood samples collected into EDTA bottle for CD4 count (cells/µl) at point of recruitment and monthly thereafter using a Partec cytometer counter (Cyflow®), among other prerequisite investigations for monitoring in ARV therapy. Each patient was prescribed combination of Highly Active Antiretroviral Therapy (HAART) of tablets of Zidovudine 300mg twice daily, Lamivudine 150mg, twice daily and Nevirapine 200mg daily first two weeks, thereafter 200mg twice daily according to the National guideline. Patients with Haemoglobin level <8g/dl, concomitant Hepatitis C or any opportunistic infections, illicit drug/alcohol use were excluded.
from the study. Biochemical monitoring was as prescribed in the National guideline for PMTCT (Nigeria). Data obtained were analysed for descriptive statistics using SPSS 17 statistical package, percentage difference of mean of the CD4 counts, stratified by trimesters of pregnancy determined and ANOVA for difference of means between groups at initiation of HAART and at 4th and 8th week respectively.

RESULTS

A total of 126 patients were recruited over the 2 years period. The overall mean age (Years), Parity and EGA (Weeks) was 29.73 ± 4.42, 1.52 ± 1.41 and 19.96 ± 4.20 respectively.

TABLE I: DEMOGRAPHY OF PATIENTS

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (Years)</td>
<td>126</td>
<td>20.00</td>
<td>40.00</td>
<td>29.73</td>
<td>4.42</td>
</tr>
<tr>
<td>PARITY</td>
<td>126</td>
<td>.00</td>
<td>5.00</td>
<td>1.52</td>
<td>1.41</td>
</tr>
<tr>
<td>EGA (Weeks)</td>
<td>126</td>
<td>12.00</td>
<td>28.00</td>
<td>19.96</td>
<td>4.20</td>
</tr>
</tbody>
</table>

In the 1st trimester group Mean0 CD4 = 408.75 cells/µL (223.70), C.I = 221.73, 595.77, Mean1 = 544 cells/µL (224.90), C.I = 355.98, 732.02, and Mean2 = 626.63 cells/µL (247.27), C.I = 419.90, 833.00. The counts in the 2nd trimester group are, *Mean0 = 367.07 cells/µL (202.67), C.I = 202.67, 328.95, *Mean1 = 489.41 cells/µL (173.23), C.I = 456.82 – 521.99, *Mean2 = 560.43 cells/µL (154.56), C.I = 531.36, 589.51. The 3rd trimester group’s parameters are as follows: **Mean0 = 540.14 cells/µL (188.86), C.I = 365.48, 714.81, **Mean1 = 607.14 cells/µL (164.22), C.I = 164.22, 455.27, **Mean2 = 650.26 cells/µL (163.85), C.I = 498.75, 801.82. The overall mean CD4 change in the 2 months of study in the three trimesters groups are 217, 192.93 and 110.12cells/µL (P = 0.088, 0.179, 0.217) respectively, while the percentages differences of Mean of the CD4 counts in between points of evaluation for each trimester are: 33.09% & 15.19% (1st trimester), 33.33% & 14.51% (2nd trimester) and 12.38% & 7.13% (3rd trimesters) respectively.

TABLE II: DISTRIBUTION OF PATIENTS BY TRIMESTERS OF PREGNANCY

<table>
<thead>
<tr>
<th>Trimesters</th>
<th>Age (Years) Mean (SD)</th>
<th>Parity Range (Median)</th>
<th>EGA (Weeks) Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Trimester</td>
<td>28.750 (4.683)</td>
<td>0 – 2 (1)</td>
<td>12.750 (0.463)</td>
</tr>
<tr>
<td>2nd Trimester</td>
<td>29.982 (4.464)</td>
<td>0 – 5 (1)</td>
<td>20.027 (3.602)</td>
</tr>
<tr>
<td>3rd Trimester</td>
<td>26.857 (2.340)</td>
<td>0 – 4 (3)</td>
<td>27.143 (0.378)</td>
</tr>
</tbody>
</table>

TABLE III: AVERAGE CD4 COUNTS OF PATIENTS AT RECRUITMENT AND MONTHLY

<table>
<thead>
<tr>
<th>Trimesters</th>
<th>Mean CD40 (C.I) cells/µL</th>
<th>Mean CD41 (C.I) cells/µL</th>
<th>Mean CD42 (C.I) cells/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>408.75 (221.73, 595.77)</td>
<td>544.00 (355.98, 732.02)</td>
<td>626.63 (419.90, 833.00)</td>
</tr>
<tr>
<td>Second</td>
<td>367.07 (202.67, 328.95)</td>
<td>489.41 (456.82, 521.99)</td>
<td>560.43 (531.36, 589.51)</td>
</tr>
<tr>
<td>Third</td>
<td>540.14 (365.48, 714.81)</td>
<td>607.00 (164.22, 455.27)</td>
<td>650.26 (498.75, 801.82)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.088</td>
<td>0.179</td>
<td>0.217</td>
</tr>
</tbody>
</table>

DISCUSSION

Highly Active Antiretroviral Therapy (HAART) typically composed of 3 antiretroviral agents from 2 drug classes has substantially reduced MTCT rates through successful suppression of HIV RNA load[1, 2]. This fact has been established in many collaborative studies and therefore formed the basis of the recommendation for the HAART prophylaxis in HIV positive pregnant women, irrespective of the CD4 count levels[1, 3, 4]. It is therefore a well-known fact that responses to antiretroviral drugs (ARVs) are both virologic (HIV RNA load) and immunologic (CD4 count).

In this study we described the pattern of CD4 changes observed in freshly diagnosed HIV positive pregnant women, across the trimesters, in our Institution, who were commenced on HAART.
(Zidovudine, Lamivudine and Nevirapine) for a period of 2 months in pregnancy.

Our findings showed numerically, but statistically insignificant, higher immunologic response in group of naïve women initiated on HAART while in the first 26 weeks of pregnancy and lesser response in those commenced in the third trimester of pregnancy (Table IV).

### TABLE IV: CALCULATED PERCENTAGE DIFFERENCE OF MEANS BY TRIMESTERS OF PREGNANCY

<table>
<thead>
<tr>
<th>Trimesters</th>
<th>% Difference of Means¹ (A1 0 - 4th week)</th>
<th>% Difference of Means² (4th - 8th week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Trimester</td>
<td>33.09%</td>
<td>15.19%</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>33.33%</td>
<td>14.51%</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>12.38%</td>
<td>7.13%</td>
</tr>
</tbody>
</table>

Key:
- CD4⁰ - CD4 at commencement of HAART
- CD4¹ - CD4 at 4 weeks of commencement of HAART
- CD4² - CD4 at 8 weeks of commencement of HAART
- CI - 95% Confidence Interval

Difference of Means¹ - Difference between initiation & 4 weeks of HAART

Difference of Means² - Difference between 4th week and 8th week of HAART

The might not be un-related to the adaptive immunology of pregnancy, whereby, elevated levels of progesterone and estrogen, greatest during the first half of pregnancy, lead to an increase in CD4⁺, CD25⁺ regulatory T cells and tolerance to alloantigens such as fetal antigens[5, 6]. The effect of pregnancy on regulatory T cells could possibly lead to a better virologic response to HAART in women first experiencing HAART during pregnancy. If this be the case, we can at least exploit this natural advantage and strive to initiate HAART in HIV positive pregnant women in the first half of pregnancy at the latest. It is incontrovertible, that opportunistic infection, AIDS related complications and consequently risks of mother-to-child infections are much less with adequate defence confer by good CD4 [7]. However, some other studies, comparing CD4 changes in HIV-1 positive pregnant women, had reported that women have lower CD4 cell counts changes during pregnancy than in the postpartum period [8]. Few studies have actually described CD4 changes in pregnant women on HAART and even in those studies, it was comparison of the changes in pregnancy with the postpartum period [9]. In our patients’ population, the mean CD4 counts of the 3rd trimester group was the highest at diagnosis and enrolment, but only improved by percentage difference of 12.38% and 7.13% in the two subsequent 4 weekly interval, compared with 33.09% and 15.19% (1st trimester group) and 33.33% and 14.51% (2nd trimester group) respectively. However, a consistent finding across the groups is progressive decline in increment in means as the weeks increase (Table IV).

Although we controlled and excluded important baseline characteristics that are associated with immune recovery (illicit drug use [10] and hepatitis C virus co-infection[11]), other factors such as baseline CD4⁺ lymphocyte count, duration of HIV infection and HIV-1 viral load, CD4⁺ lymphocyte count nadir [12] could not be excluded. Some other inherent limitations preclude causal inferences from this study. First, being that the study is observational in nature. Also, the patients are not evenly distributed across groups, third is the time limit conferred by the duration of pregnancy and lastly, perhaps most important is the inability, due to limited resources in our setting, to concurrently assess the viral load and correlate with the CD4 count. It must however be stated that WHO and National guideline, due to this resources limitation recommended CD4 counts for response monitoring in HIV patient on ARV[13, 1].

In this study, we recorded 2 perinatal deaths and 4 HIV neonatal infections by the 6th month of follow-up (vertical transmission rate of 3.17%), 3 of which occurred in the women enrolled in the 3rd trimester and 1 in the 2nd trimester, while both perinatal deaths were due to severe asphyxia. All the women in whom neonatal infections were recorded breastfed their babies and were continuing prophylactic HAART.

It is however, noteworthy, that possibly because of exclusion of other co-infections - HCV and other opportunistic infections, as well as drug and alcohol abuse, most of the patients had above 400 CD4 from 4th week of HAART initiation.
With the above noted, this study found a better immunologic response among HIV positive, ARV naïve women who started HAART during the first 26 weeks of pregnancy compared to women who started HAART in the last 13 weeks, though a causal relationship could not be established due to the low power of this study. However, in our opinion, this might pose great clinical challenges in our environment where late antenatal care booking is the norm. At the moment, HAART’s response monitoring in our environment is limited to Immunologic parameter (CD4 count) only, with just very few research centres having the capacity to undertake virologic monitoring.

It is hereby summated that there might be better immunological response in ARV naïve HIV positive pregnant women when commenced on HAART in the first 26 weeks of pregnancy, as progressive decline in CD4 increase was observed as the pregnancy advanced across the trimester, with the least immunological response and more neonatal infection observed in 3rd trimester group. Public health enlightenment should utilize this information and efforts should be directed to the initiation of HAART prophylaxis before end of 2nd trimester of pregnancy. Larger studies with concurrent viral load assessment are however recommended to further explore causal relationship of these factors.

CONFLICT OF INTEREST NOTIFICATION:
We declare that we have no conflict of interest; no funding/grant was received for this study and no commercial relationship.

Abstract presented at the 45th Annual Scientific Conference & General Meeting of The Society of Gynaecology and Obstetrics of Nigeria (SOGON), held in Ibadan, Nigeria, 22nd – 26th November 2011.

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ISOLATION OF ENTEROVIRUS FROM FEACAL SAMPLES OF PATIENTS WITH DIABETES MELLITUS IN MAIDUGURI, NIGERIA.

1,2 Oderinde, B.S.*, 1,2 Baba M.M., 1 Nwodo, M.U., 2 Ladan, J., 2 Musa, B.J., 2 Ghamba, P.E., 2 Akinola, M.T. & 2 Kadai, A.M.

1. Department of Medical Laboratory Science, College of Medical Sciences. University of Maiduguri, P.M.B 1069 Maiduguri, Borno State. Nigeria;
2. World Health Organization National Polio / ITD Polio laboratory, University of Maiduguri Teaching Hospital, P.M.B 1414 Maiduguri, Borno State. Nigeria.

Correspondence: Oderinde B.S. Department of Medical Laboratory Science, College of Medical Sciences. University of Maiduguri, P.M.B 1069 Maiduguri, Borno State. Nigeria. E-mail: sojihunter@yahoo.com

ABSTRACT

In this study, 150 patients were recruited out of which 63(42%) were male while 87(58%) were female subjects. Patients with type 1 diabetes were 2(1.3%), those with type 2 were 142(94.7%) while those with GDM were 4(4%). Only one sample from type 2 was positive by virus isolation and identified to be Echovirus 1 and 21 by microneutralization tests as described in WHO polio laboratory manual, 2004. It has been demonstrated that enterovirus infections were significantly more common in recently diagnosed diabetic patients, compared to control subjects. The question if enterovirus could cause beta cell damage and diabetes mellitus has become more and more relevant when recent studies have provided new evidence supporting this scenario especially in type 1 diabetes. This is an important issue since it opens the possibility to develop new, preventive and therapeutic strategies to fight the disease. The purpose of this study is to investigate if enterovirus can be isolated from the stool samples of diabetic patients as a study.

Key words: Isolation, enteroviruses, faeces, diabetes mellitus, patients,

INTRODUCTION

Diabetes mellitus or diabetes is a lifelong chronic disease in which there is high level of sugar in the blood which results in symptoms such as blurry vision, excess thirst, fatigue, frequent urination, hunger and weight loss (1). There are three major types of diabetes mellitus: Type 1 diabetes mellitus can occur at any age but is most frequently diagnosed in children-teen or young adults. In this disease the body makes little or no insulin. Type 2 diabetes mellitus which makes up most diabetes cases. It most often occurs in adults (2). It results from insulin resistance; a condition in which cells fail to utilize insulin properly. Gestational diabetes: when pregnant women who have never had diabetes before have a high blood glucose level during pregnancy.

Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases (3). Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double (4). Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030. The increase in incidence in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented (4).

The infection with enteroviruses seems to be linked to the induction of islet-cell autoantibodies as well as to the expression of interferon-α. Both of these events are connected with islet-cell destruction (5). It has become increasingly important to establish the nature of the infecting virus in the early stages of diabetes and seems a number of viruses of the coxsackie or echovirus type are involved, although the nature of the nucleotide sequences responsible for diabetogenicity remains elusive. Viruses have long been considered a major environmental factor in the aetiology of type 1 diabetes mellitus and recent work has greatly confirmed this role and now evident that enteroviral infections accompany the onset of diabetes in young people. It is only recently that clearer role of this virus in the aetiology of diabetes began to emerge despite large volume of work devoted to them. Diabetes
Enterovirus and type 1 diabetes

An extension of the study to adults with type 2 diabetes showed that a large proportion of the same age group. Unlike type 1 diabetes, type 2 diabetes usually starts in adults and is associated with obesity. The beta cells are not destroyed in this disease but their ability to make insulin is compromised. The way that enteroviruses might contribute to the development of type 2 diabetes has not been established but it is known from laboratory studies that an entero viral infection of beta cells reduces their ability to release insulin. It is possible that in people who are obese (where there is a greatly increased demand for insulin secretion) a reduction of beta cell function, secondary to entero viral infection, may be sufficient to trigger type 2 diabetes - although more research is required to confirm this.(18).

Another recent study to be published in www.phgfoundation.org/news/4515/ proposed link between T1D and enterovirus infection which reports the detection of enteroviruses in pancreatic tissue from about 60% of 72 paediatric patients with T1D, but very few of the samples of tissue from 50 children without the disease (20). This research also found that 40% of adults with type 2 diabetes showed signs of enteroviral infection of their pancreatic beta cells, compared with only 13 percent of non-diabetic (control) adults of the same age group. The authors propose that vaccination against entero viral infection in childhood could reduce the incidence of both common forms of diabetes.

MATERIALS AND METHODS

Study area:

Stool samples were collected from adults who visited University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria for medical attention and follow up treatments. The hospital is a tertiary...
health institution located in Borno State, Nigeria and serves as a referral health centre for six states (Adamawa, Bauchi, Borno, Gombe, Taraba and Yobe,) in northeastern Nigeria and neighboring African countries (Chad to the northeast, Niger to the north and Cameroon to the east). Based on the 2006 provisional census figures, Borno State has a population of 4,151,193 and a population density of approximately 60 inhabitants per square kilometer. The state has an area of 61,435 sq. km (the largest state in Nigeria in terms of land mass).(21).

Study population:

The Ethical Committee of University of Maiduguri Teaching Hospital, Maiduguri gave approval to the study protocol. Only patients with confirmed diabetes were recruited for the study. After obtaining informed consent from those subjects, willing participants were enrolled consecutively in the study. Demographic data were collected.

History of the subjects studied:

The record of the subjects tested was not available but information in this regard was obtained verbally. However,

Sample collection:

A total of 150 stool samples were collected from the subjects studied between January and February 2012. Clean universal containers are given to each patient to be returned the next day with fresh fecal sample (about 2g or the size of an adult thumb). The stool samples were properly labelled and transported to the World Health Organization National Polio Laboratory, Maiduguri for storage and analysis. The entire stools were stored at -20°C until tested.

Virus Isolation and Identification / Microneutralization tests for Non Polio enteroviruses.

The methods adopted for virus isolation and microneutralization for identification of isolates were described in WHO Polio Laboratory Manual (2004) and the supplemental manual of (2006) for the New Algorithm Technique currently used for poliovirus isolation. Fecal samples were pretreated with chloroform and phosphate buffered saline and centrifuged at 3000 rpm for 30 minutes, 200ul of the supernatant was inoculated onto healthy monolayer of L20B and Rhabdomyosarcoma (RD) cell lines (Source: Centre for Disease Control and Prevention, Atlanta, USA) in maintinance medium (Eagle’s MEM Supplemented with 2% Fetal Calf Serum (FCS). The cells were seeded 48 hours prior to inoculation with growth medium (Eagle’s MEM supplemented with 10% FCS). The inoculated monolayers were incubated at 36°C and observed daily for the characteristic enterovirus cytopathic effects (CPE) of rounded refractile cells and detaching from the surface of the tube. The tubes with CPE up to 75% and above were harvested and kept at -20°C to be passaged to a fresh monolayer of the second cell line. While those negative after 5 days of incubation at 36°C were repassaged on the same cell line. Tubes showing no CPE after the second five days were considered as negative. With the New Algorithm Technique, positive isolates usually end in RD cell line because virus titre seems higher in RD than L20B. Positive samples on RD cells were passaged onto L20B, the selective cell line for poliovirus. Identification of isolates on RD cells was carried out by microneutralization technique using antisera raised in horse against coxsackie and echoviruses prepared by the National Institute of Public Health and Environment (RIVM), Netherlands.

RESULTS

Students SPSS version 16 was used for the computation and the results obtained are below:

<table>
<thead>
<tr>
<th>Types of Diabetes</th>
<th>Number tested</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>Type 2</td>
<td>142</td>
<td>94.7</td>
</tr>
<tr>
<td>Gestational diabetes (GDM)</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 presents the number and percentages of diabetic patients with the 3 types of diabetes. It is shown that type 2 is the most predominant with 142(94.7%), followed by gestational diabetes (GDM) in 6(4%) pregnant women and type 1 with lowest with 2(1.3%) patients.

Table 2 represents the age range of the 150 diabetic patients with number involved. Out of 150
samples, 2(1.3%) were less than 20 years, 6(4.0%) were within the age group of 21-29, 17(11.3%) were within 30-39, 49(32.9%) within 40-49, 50(33.3%) within 50-59, while 26(17.3%) were within 60 and above. It can be inferred that most diabetic onset starts the age of fourties. In addition the highest number of patients falls in the fifties as a result of ageing factors.

**TABLE 2. AGE RANGE DISTRIBUTION OF DIABETIC PATIENTS N=150.**

<table>
<thead>
<tr>
<th>Age groups (yrs)</th>
<th>Number (150)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>21 – 29</td>
<td>6</td>
<td>4.0</td>
</tr>
<tr>
<td>30 – 39</td>
<td>17</td>
<td>11.3</td>
</tr>
<tr>
<td>40 – 49</td>
<td>49</td>
<td>32.7</td>
</tr>
<tr>
<td>50 – 59</td>
<td>50</td>
<td>33.3</td>
</tr>
<tr>
<td>60 – 69</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>≥ 70</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>150</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

**TABLE 3: DISTRIBUTION OF DIABETIC PATIENTS BY DURATION / ONSET**

<table>
<thead>
<tr>
<th>Duration / Onset (yrs)</th>
<th>Number (150)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 5</td>
<td>98</td>
<td>65.3</td>
</tr>
<tr>
<td>6 - 10</td>
<td>32</td>
<td>21.3</td>
</tr>
<tr>
<td>11 - 15</td>
<td>11</td>
<td>7.3</td>
</tr>
<tr>
<td>16 - 20</td>
<td>4</td>
<td>2.7</td>
</tr>
<tr>
<td>21 - 25</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>26 - 30</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>150</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 3 shows the distribution of patients with the duration of onset of diabetes in years. Out of 150 patients whose samples were used, 98(65.3%) have had diabetes for less than or equal to 5 years, 32(21.3%) for about 6-10 years, 11(7.3%) for about 11-15 years, 4(2.7%) for about 16-20 years, while 2(1.3%) have had it for about 26-30 years. The highest figure obtained with onset of 1-5 years might be due to increased publicity and awareness of the people. This reflects increase in number of diabetic patients with decreased year(s) of onset that seeks medical attention.

**DISCUSSION** Enteroviruses have long been considered a major environmental factor in the aetiology of diabetes mellitus (5). These viruses have been detected from diabetic patients more often than from control subjects and they can infect beta cells in cell culture and induce diabetes in animal models (23). A research conducted by Salminen (2004) in Finland on children between 6-18 years, revealed that enterovirus can be isolated in stool samples.

In this work, out of 150 samples collected 63(42%) were male while 87(58%) were female subjects also, subjects with type 1 were 2(1.3%), type 2 were 142(94.7%) and GDM were 6(4%), this is owing to the fact that type 2 is the most common type which is due primarily to lifestyle modification, genetics and viruses (25). The only positive sample was from a male subject with type 2 diabetes and fell within the age bracket of 50-59 with duration of within 11-15 years. There is no related literature to justify why it was male and within that age group and the given duration.
The Echovirus 1 and 21 that was isolated from the positive case might not be responsible for the aetiology of Type 2 diabetes as this may not be unconnected with the poor hygiene standards commonly observed in most communities as previously reported (26). In addition this serotypes were not among the ones isolated in aetiology of acute flaccid paralysis in northern Nigeria of which this study area was inclusive (27) meaning that further researches need to establish echovirus in aetiology of Type 2 diabetes. However, the fact that no absolute association has been identified with certain viral strains or even viral geniuses or families indicates that, if Type 1 diabetes is indeed caused by viruses, multiple infectious strains may result in the same disease phenotype (28). In conjecture, there is need for further researches at molecular level to establish this fact or probably the unbiased metagenomics techniques.

The Gestational Diabetes Mellitus (GDM) came into consideration as maternal enterovirus infection is a risk factor for Type 1 diabetes in the exposed offspring (17). This emphasizes the fact that pregnant women needs to be screened for enterovirus infection to carry out preventable measures before delivery of the offspring. In addition, there is need to implement health education in Antenatal clinics in this regards. There is no documented evidence regarding the association of enterovirus in the aetiology of Type 2 diabetes which form 94.7% of the patients tested, and no virus isolation was made. Considering the duration of the onset of the infection, 0-5 range has the highest with 68.8% which might not be unconnected with the increased awareness of the people even at the grass root level. However, more publicity is still required with the provision of basic care at the Primary Health Care facility which is supposed to be made available at the closest reach of every Nigeria. However, the timing cannot be underplayed as a component determining virally mediated modulation of autoimmunity appears to be the time at which infection occurs during the pre-diabetic phase e.g. type 1 diabetes is enhanced in 8 week old NOD mice infected with Coxsackie B4, infection of younger mice has no effect on disease outcome which suggests that the status of autoimmune progression is a crucial determinant in the diabetogenic potency of the virus (29). The highest prevalence was within 50-59 years with 33.3%, followed by 40-49 years with 32.7% which of course might be due to ageing factor.

In conclusion, these patients samples based on retrospective study showed that they are diabetic patients. Only 1 (0.6%) of the patient with type 2 diabetes stool sample were positive while 99.3% were negative. Based on this result it cannot be concluded that enteroviruses do not cause diabetes mellitus, various factors has to be considered such as; Type of diabetes, patients residence / environment, timing of infection and subjects hygiene standards enteroviral destruction of β-cells of the pancreas occurs years before clinical symptoms of diabetes appears. If samples were collected at onset of clinical symptoms then most of this virus could be isolated. In addition, there is need to establish the role of echovirus in the aetiology of diabetes by advanced unbiased metageomics technique, which will give a clearer information and data.

ACKNOWLEDGEMENTS

We thank the WHO for provision of technical support to the laboratory and management of University of Maiduguri Teaching Hospital (UMTH) for their contribution to the study by guiding and giving us ethical clearance.

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RUBELLA IgG ANTIBODY AMONG NIGERIAN PREGNANT WOMEN WITHOUT VACCINATION HISTORY

M. O. Adewumi, R. B. Olusanya, B. A. Oladunjoye, J. A. Adeniji
Departments of Virology, College of Medicine, and Microbiology University of Ibadan; Correspondence: Department of Virology, College of Medicine, University College Hospital, Ibadan, Nigeria. Telephone: +234 806 022 6655 E-Mail: adewumi1@hotmail.com

Abstract
Rubella is a vaccine-preventable viral infection, its aetiologic agent; rubella virus was identified as human teratogen capable of causing a spectrum of birth defects described as congenital rubella syndrome (CRS). Despite the availability of safe and effective vaccines, significant proportion of the population remains susceptible to rubella infection in developing countries. More significantly, such developing countries including Nigeria have not demonstrated adequate commitment to preventive vaccination; a panacea for intervention. Consequently, this study was designed to determine the prevalence of anti-rubella IgG among pregnant women to ascertain the proportion of susceptible population. A total of 273 consenting rubella vaccine naïve antenatal clinic attendees aged 15-42 years (Median age = 28 years) were randomly selected and their sera analyzed for qualitative and quantitative anti-rubella IgG detection. Overall, 244/273 (89.4%) pregnant women enrolled in this study had protective level (Titre = >10 IU/mL) of anti-rubella IgG (Median Titre = 165 IU/mL; Range = <10 - >250 IU/mL), while, 29/273 (10.6%) of the study population lack protective antibody titre (OD = <10 IU/mL). Results confirm previous reports of exposure, infection, and continuous circulation of rubella virus in Nigeria. It emphasizes the need for improved and continuous surveillance for rubella and CRS cases, prompt vaccination of vulnerable populations, and evaluation of health policies to achieve immunization and ultimately ensure control/elimination of rubella virus in Nigeria and beyond.

Keywords: Rubella, Pregnancy, Antibody, Congenital Rubella Syndrome, Nigeria

INTRODUCTION
Rubella is a vaccine-preventable viral infection of the skin and lymph nodes often culminating in a mild rash. The aetiologic agent is a positive sense single-stranded RNA rubella virus in the family Togaviridae [1, 2]. The virus has been identified as a human teratogen capable of causing a spectrum of birth defects often collectively referred to as congenital rubella syndrome [3, 4, 5] or death [6, 7] of a developing foetus, especially if the viral infection is acquired in the early months, that is first trimester of pregnancy [2, 8, 9, 10].

In spite of the availability of a safe and effective vaccine against rubella, an estimated over 100,000 infants are born with congenital rubella syndrome annually [6, 11, 12]. Additionally, approximately 3 to 23% of adults remain susceptible to rubella virus infection in various countries and areas, although studies have shown that a large proportion of unimmunized populations in areas where rubella is endemic are infected and become seropositive before puberty [13, 14, 15, 16].

Dwyer et al., (2001) noted that about 10-25% of non-immunized women of childbearing age are susceptible to rubella virus infection [17]. Furthermore, studies have shown that 80-90% of babies born to women infected with rubella virus during the first trimester of gestation experience birth defects [9, 18]. Humans are the only known reservoir for rubella virus; hence, its maintenance requires continuous access to a susceptible population. Equally, elimination of rubella and CRS with an effective vaccination program in some countries [19] is an evidence of achievable intervention plan for rubella virus and the disease.

Despite the reality of preventive rubella vaccine, routine screening and preventive vaccination against rubella virus for women and children have not been incorporated into the antenatal care and National Program on Immunization (NPI) in Nigeria. Also, rubella infection and CRS are not reportable diseases in the country. Therefore, to achieve intervention in Nigeria and beyond, there is the need to assess current situation of rubella infection especially among at risk population. Such information is essential to demonstrate the need for government to support preventive vaccination, especially among women of childbearing age, and surveillance for CRS to
facilitate prompt intervention. Therefore, to achieve the aforementioned, this study was designed to determine the prevalence of anti-rubella IgG among pregnant women in order to ascertain the proportion of susceptible population.

MATERIALS AND METHODS

STUDY LOCATION

This study was carried out among pregnant women attending Ade-Oyo Maternity Hospital in Ibadan, south western, Nigeria. The hospital is located in a densely populated centre of the city and serves pregnant women of varied educational status from different parts of the city. The ante-natal clinic records an average of 380 + 20 new patients per week.

ENROLMENT OF THE STUDY POPULATION

Participants for this study were enrolled in July, 2010. After approval was obtained from the Hospital Management Board, each prospective participant was counselled, and only consenting clinic attendees were enrolled for the study. During the enrolment period, the ante-natal clinic was visited five times. Blood samples were collected from a total of 273 consenting pregnant women (Median age = 28 years; Range = 15 - 42 years) at the point of registration for the ante-natal clinic. The participants were mostly women of low educational status, thus could not provide any proof or record of rubella vaccination. Rubella vaccines are currently available in Nigeria at a cost, thus limited to the elites who appreciate the importance of preventive vaccination.

SAMPLE COLLECTION, PREPARATION AND STORAGE

About 5 millilitres of blood specimen was collected by venepuncture from each consenting pregnant woman into a sterile container free of anticoagulants or preservatives. Each specimen was labelled with the date of collection and laboratory identity number. Samples were transported to the laboratory immediately in a cold box with frozen ice packs to achieve condition of about 4-8°C. Serum samples were separated by low-speed centrifugation at 500 x g for 5 minutes, or direct removal of the serum using a sterile disposable pipette after retraction of the clot. The serum was transferred into labelled sterile cryovials and stored at -20°C until ready for analysis.

LABORATORY ANALYSIS

The laboratory analysis was carried out in the Department of Virology, College of Medicine, University College Hospital, Ibadan. The samples were analyzed for qualitative and quantitative detection of anti-rubella IgG antibody using DIA.PRO® Diagnostic Bioprobes s.r.l. (Sede legale: Via Lucio Giunio Columella, 31-20128-Milano) Enzyme Immunoassay in accordance with the manufacturer’s description. The test kit has diagnostic sensitivity and specificity performance of >98%.

STATISTICAL ANALYSIS

Demographic features of the study population were described. Also, results of the study were analyzed using the statistical package for the social sciences (SPSS) version 15.0 windows, and p-value <0.05 was considered statistically significant.

RESULTS

Overall, 244/273 (89.4%) pregnant women enrolled in this study had protective level (Titre = >10 IU/mL) of anti-rubella IgG (Median Titre = 165 IU/mL; Range = <10 - >250 IU/mL), while, 29/273 (10.6%) of the study population lack protective antibody titre (OD = <10 IU/mL). Nine out of the twenty-nine (31.0%) women in the later category had no detectable anti-rubella IgG antibody (OD < 0 IU/mL). Analysis by age shows highest and lowest rates of seropositivity in age groups >40 and 31-40 years respectively (Table 1). However, no statistically significant difference (p=0.906) was recorded in the rates of seropositivity to rubella virus infection by age.

DISCUSSION

Previous studies [20, 21, 22, 23, 24] have reported varied prevalence rates of anti-rubella IgG among Nigerian population including pregnant women. More significantly, in 2002 Bukbuk et al., [25] in a study among non-immunized pregnant women in Maiduguri, north eastern Nigeria reported 54.1% prevalence of rubella-specific IgG antibody. Consequently, detection of anti-rubella IgG in 89.4% of pregnant women without history of rubella vaccination in this study corroborates findings from previous studies in the country [20, 21, 22, 23, 24, 25]. Furthermore, detection of anti-rubella IgG among rubella pregnant women without vaccine history is an indication of previous exposure and subclinical or clinical infection by rubella virus. Therefore, this confirms continuous circulation of rubella virus in a country where preventive rubella vaccine is only available for the informed adult population at a cost. Additionally, it confirms earlier reports [24, 26, 27] that rubella virus may have been circulating continuously in Nigeria but for paucity of data on its burden.
TABLE 1: RUBELLA IgG ANTIBODY AMONG PREGNANT WOMEN OF DIFFERENT AGE GROUPS ATTENDING ANTE-NATAL CLINIC IN IBADAN, NIGERIA

<table>
<thead>
<tr>
<th>AGE (YEAR)</th>
<th>NUMBER TESTED (%)</th>
<th>NUMBER POSITIVE (%)</th>
<th>NUMBER NEGATIVE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>30 (11.0)</td>
<td>27 (90)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>21-30</td>
<td>170 (62.3)</td>
<td>152 (89.4)</td>
<td>18 (10.6)</td>
</tr>
<tr>
<td>31-40</td>
<td>69 (25.3)</td>
<td>61 (88.4)</td>
<td>8 (11.6)</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>4 (1.5)</td>
<td>4 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>273 (100)</td>
<td>244 (89.4)</td>
<td>29 (10.6)</td>
</tr>
</tbody>
</table>

Highest rates of seropositivity to rubella observed among age group >40 years supports earlier suggestion that longer period and probably higher frequency of childbearing and nursing experienced in such age group may predispose them to greater risk of exposure and infection. Lack of protective rubella IgG antibody in 29/273 [10.6%] of the study population and more significantly lack of detectable antibody in 9 out of the 29 women suggest existence of susceptible population for rubella virus maintenance in the community. This finding confirms earlier reports [26, 28] that despite the development and administration of effective vaccines for prevention and control of rubella virus infection since 1969 and prevention or elimination of the causative agent in many developed countries, cases of rubella virus infection and CRS are still being reported among diverse groups in Nigeria.

However, since humans are the only known reservoir for rubella virus, maintenance of rubella requires continuous access to a susceptible population. Therefore, an enhanced immunization programme aimed at ensuring high level of herd immunity would facilitate the control of rubella epidemics [29]. Furthermore, findings from this and previous studies in the country indicate that Nigeria has in its hands, an opportunity to eliminate the virus since the burden is low and the definite susceptible population is defined.

Rubella naive individuals are susceptible to infection, thus constitute risk of transmission and maintenance of a vaccine-preventable virus infection in the community and the world at large. Prevention or elimination of rubella virus infection has been achieved in many developed countries with the introduction of preventive vaccine. Therefore, to facilitate prompt and effective virus elimination in the country, immediate introduction of preventive rubella vaccination to susceptible population is essential.

In conclusion, results of the study emphasize the need for establishment of an improved and continuous surveillance network for rubella infection and CRS cases; prompt vaccination of vulnerable population and periodic evaluation of health policies to guarantee immunization. Thus, to facilitate prompt elimination of rubella we recommend the establishment of health policy that promotes: (a) free vaccination programmes for children and adults of childbearing age (b) evaluation of available vaccines to ascertain their potency prior recommendation for vaccination (c) review of antibody response in randomly selected individuals post vaccination, and (d) rubella virus and CRS surveillance.

ACKNOWLEDGEMENTS
We acknowledge with gratitude, the support of the Medical Director and the entire Hospital Management Board as well as the cooperation by all participants. Author’s contributions: MOA and JAA were involved in the conception and design of the study. MOA, RBO and BAO were involved in sample and data collection, analysis and interpretation of results. Manuscript preparation was by MOA, while revision of the manuscript was by MOA and JAA.
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