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ORIGINAL ARTICLE

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INVASIVE BEHAVIOUR AND DEPOLARIZATION EFFECT OF *PSEUDOMONAS FLUORESCENS* ON RAT CEREBELLAR GRANULE NEURONS

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Previous studies have shown that *Pseudomonas fluorescens* exerts cytotoxic effects on neurons and glial cells. In the present work, we investigated the time course effect of *Pseudomonas fluorescens* MF37 and of its lipopolysaccharide (LPS) on cultured rat cerebellar granule neurons. The kinetics of binding of *P. fluorescens* to cerebellar granule neurons is identical to that of cortical neurons but the binding index is lower, suggesting the presence of a reduced number of binding sites. As demonstrated by measurement of the concentration of nitrites in the culture medium, *P. fluorescens* induces a rapid stimulation (3 h) of the nitric oxide synthase (NOS) activity of the cells. In contrast, LPS extracted from *P. fluorescens* requires a long lag phase (24 h) before observation of an activation of NOS. Measurement of the resting membrane potential of granule neurons showed that within 3 h of incubation, there was no difference of effect between the action of *P. fluorescens* and that of its LPS endotoxin. Two complementary approaches allowed us to demonstrate that *P. fluorescens* MF37 presents a rapid invasive behaviour, suggesting a mobilisation of calcium in its early steps of action. The present study reveals that *P. fluorescens* induces the sequential activation of a constitutive calcium dependent NOS and that of an inducible NOS activated by LPS. Ours results also suggest that *P. fluorescens* cytotoxicity and invasion are not mutually exclusive events.

Key words: Cytotoxicity, Lipopolysaccharide, Patch-clamp, Invasion, *Pseudomonas fluorescens*

INTRODUCTION

Pseudomonas fluorescens, a bacterium closely related to the opportunistic pathogen *Pseudomonas aeruginosa* is considered to be as common as this species in the environment [1]. Few years ago, *P. fluorescens* was found to be responsible for 60% of the cases of post transfusion septicaemia in United Kingdom [2]. The involvement of *P. fluorescens* in infections of the central nervous system (CNS) in human remains controversial because of the difficulty in identifying the strains present in hospital [3]. However, this bacterium is known to cause local facial infections by using routes of penetration into the CNS identical to those of *P. aeruginosa* [3]. In addition, it is well established that *P. fluorescens* provokes CNS infections in fish [4] and express binding proteins for γ-aminobutyric acid, the main inhibitory neurotransmitter of the CNS [5, 6].

We have previously shown that *P. fluorescens* can bind to glial cells [7] and that its lipopolysaccharide (LPS) modulates potassium channels in target cells [8]. The dose-related effect of the LPS from *P. fluorescens* has been determined on glial cells and cortical neurons using a morphological approach [9]. Also, we have demonstrated that within 24 h, the LPS can induce the expression of a nitric oxide synthase (NOS) associated with apoptosis [9]. However, until now the time course of the cytotoxic effect of *P. fluorescens* on neurons has not been studied in details since this work requires a cell population to investigate the effect of both intact living bacteria and LPS on biochemical indicators

of cytotoxicity and on the plasma membrane potential.

In addition, since membrane ionic currents play a central role in the adhesion of bacteria to the target cells and on the internalisation activity [10], it appeared essential to correlate the cytotoxic activity of *P. fluorescens* to its binding and invasive potential.

In the present study, we selected a homogeneous neuronal cell line, cerebellar granule neurons, to compare the kinetics of the cytotoxic action of *P. fluorescens* MF37 and its LPS and the relative effect of the bacterium and its LPS endotoxin on the membrane currents. The results are discussed in regard to the activation of the different NOS expressed in neurons and to the invasive behaviour of the bacterial strain.

MATERIAL AND METHODS

Bacterial culture and LPS extraction

The strain MF37 of *Pseudomona fluorescens* is a natural rifampicin resistant mutant of the psychrotrophic strain MF0 isolated from crude milk [11]. *P. fluorescens* MF37 was grown at 28°C in Luria Bertani (LB). Just before the experiments bacteria in early stationary phase were harvested by centrifugation (6000 rpm, 4 min, 20°C) and resuspended in culture medium bathing neurons without antibiotics or antimycotics. The LPS from *P. fluorescens* was purified as previously described [12]. The degree of purification of the LPS and the absence of contaminant protein was controlled by capillary electrophoresis analysis of the extracted molecule following the technique of Picot *et al* [13]

Culture of neurons

Cerebellar granule neurons were obtained from 6-8 day old rats. The cerebellum was mechanically dispersed in culture medium consisting of DMEM/Ham's medium (3:1) supplemented with 10% foetal calf serum, 2 mM glutamine, 5 µg/ml insulin, 100 µg/ml transferrin, 20 µM putrescine, 1 mM sodium pyruvate, 30 nM sodium selenite, 25 mM KCl, 20 nM progesterone and containing 1% antibiotic-antimycotic solution. The suspension was filtered on a sterile 82 µm nylon filter to remove the remaining tissue fragments. Neurons were plated on glass coverslips at a density of 1.5×10^6 - 8×10^6 cells/ml after the experiments.

Adherence assays

For the adherence assays, the culture medium of neurons was replaced by medium without antibiotics and antimycotics, and the bacterial suspension was added at a concentration of 10^6 CFU/ml which roughly corresponds to that considered as a pathogenic dose [14]. The cells were fixed for 5 min in methanol and incubated in Coomassie blue 0.5% in methanol/water (V/V) and counterstained for 15 min in a Giemsa solution. Culture slides were rinsed 3 times for 5 min in methanol/water (1:1) and mounted under glass slides in phosphate buffer (PB; 0.1 M, pH 7.4) – glycerol (1:1). The binding index was determined by counting the number of bacteria adherent to the cytoplasmic membrane of the cells. Observations were done under an Axiovert S100 optical microscope (Zeiss, Oberkochen, Germany) equipped with an Olympus SC35 photographic system.

Determination of the nitric oxide synthase (NOS) activity

Nitrite ions (NO^{2-}), considered as representative of the activation of nitric 0oxide synthase (NOS) involved in the apoptotic process of neurons, were assayed using a technique derived from the Griess colorimetric reaction. The experiments were performed using neurons plated at high density (8 x 10^6 cells/ml) and after 7 days of culture in vitro (DIV=7). The assays were carried out using living bacteria (*P. fluorescens* 10^6 CFU/ml) and LPS from *P. fluorescens* (200ng/ml). Control studies were performed by the use of; i) bacteria incubated for the same period in culture medium for neurons but in the absence of the cells, and ii) an extraction buffer made following the same protocol used for the extraction of LPS but in the absence of bacteria. The intra- and inter-assay coefficients of variation were lower than 3 and 8% respectively

Measurement of the resting membrane potential (RMP) of neurons

Electrophysiological recordings were performed at room temperature (20-22°C) using the patch-clamp technique [15] in the whole-cell configuration in current-clamp mode. Recordings were made with a patch-clamp amplifier (Axopatch 200B, Axon Instruments Inc, USA). The culture medium was replaced by a bathing solution (pH = 7.4) containing 145 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose. Cells were allowed to adapt to the new medium for 15 min. The patch-pipette (3-5 MΩ, Harvard apparatus) was filled with a solution of 100 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA and 10 mM HEPES, (pH = 7.4). Data acquisition was performed with a computer-controlled interface using pclamp software version 8.1 (Axon Instruments, USA).

Tests of invasiveness

The invasive behaviour of *P. fluorescens* in cerebellar granule neurons was investigated using two complementary approaches, the microscopic acridine orange internalisation assay and the gentamicin survival assay.

In the acridine orange assay, which is based on the technique described by Goldner *et al* [16], neurons exposed to bacteria (10^6 CFU/ml) for 4 h were incubated with 0.01% acridine orange in Gey's solution for 45 s at room temperature. The samples were rinsed with fresh culture medium and then stained with 0.05% crystal violet in culture medium for 45 s. After rinsing, the slides were mounted with culture medium and immediately observed or photographed. Acridine orange enters freely into the neurone cells and stains both extracellular and intracellular bacteria. Crystal violet, which does not penetrate into the neurons, quenches the extracellular acridine fluorescence. Thus the only bacteria that can be visualised are those present inside the cells.

The gentamicin survival assay is a technique adapted from that used to quantify *Pseudomonas aeruginosa* invasion in epithelial cells [17]. Briefly, neurons were exposed to bacteria (10^6 CFU/ml) for 4 h in culture medium without antibiotics and antimycotics. The bacterial concentration was controlled by measurement of optical density and plating. At the end of the incubation period, cultured neurons were rinsed 3 times with fresh medium to remove free bacteria. Control cultures were immediately treated with 1 ml Triton X100

in PB (0.1% v/v). After plating and counting, the total number of bacteria present at the surface and inside the cells was determined. To determine the number of intracellular bacteria, after incubation with *P. fluorescens* and rinsing, the cultures were exposed to gentamicin (300µg/ml) for 1 h. The cultures were ,then rinsed 3 times with 1 ml fresh culture medium to remove residual gentamicin and the cells were lysed with 1 ml Triton X100 in PB (0.1% v/v) before plating and counting. A blank was performed by incubating *P. fluorescens* MF37 for 1h with gentamicin (300µg/ml) to verify the effect of the antibiotic on the viability of the micro-organism. Colonies corresponding to living bacteria were counted after 2 days of culture at 28°C. Each measure was performed in triplicate.

Statistics

All data were calculated as mean ± S.E.M. Statistical analysis were evaluated by use of the unpaired Student's *t* test. The curves were fitted through polynomial regression equations using Sigma Plot V.

RESULTS

Adherence of P. fluorescens to cerebellar granule neurons and morphological effects

Cultured rat cerebellar granule neurons at day 7 were used. This culture period was previously found to correspond to the minimal duration allowing the cerebellar granule neurons to reach their mature state [18]. As illustrated in Fig. 1 A, adherence of bacteria to neurons occurred rapidly. After a 5-h period of incubation, the adsorption index reached 3.03 ± 0.14 bacteria/neuron. The adherence of bacteria to the plasma membrane was followed by marked morphological changes at the level of both the cell body and neurites. In the absence of bacteria, mature cerebellar granule neurons typically exhibited clear T-shapes and possessed several dendrites and thin axons with well defined cytoplasmic limits (Fig. 1 B). In contrast, in the presence of bacteria, a large number of vacuoles were observed in the cell body (Fig. 1 C). Moreover, a pronounced leakage of the cytoplasmic content was detected.

Kinetics of the stimulation of the NOS of cerebellar granule neurons by P. fluorescens and its LPS

The effect of *P. fluorescens* MF37 (10^6 CFU/ml) and of its lipopolysaccharide (LPS, 200 ng/ml) on the nitric oxide synthase (NOS) activity of cerebellar granule neurons was determined after 3, 12 and 24 hours of incubation. In the absence of treatment, the basal level of NO^{2-} measured in the medium was under the detection limit of the assay and remained undetectable after 3, 12 or 24 h of culture. A 3 hour incubation of neurons with *P. fluorescens* MF37 induced a rapid increase in the concentration of NO^{2-} that reached 22.3 ± 0.4 µg/ml (Fig. 2A). As indicated by the concentration of nitrite ions detected in the medium after 12 and 24 h of incubation (21.2 ± 0.9 µg/ml and 21.1 ± 1.2 µg/ml, respectively), the effect of living bacteria was already maximal after 3 h of incubation.

The kinetics of action of the LPS extracted from *P. fluorescens* was totally different (Fig. 2B). When cerebellar granule neurons were incubated for 3 or 12 h with LPS, the concentration of nitrite ions in the culture medium remained below the detection limit of the assay and apparently unchanged. It is only after 24 h of incubation that a rise in the concentration of NO^{2-} was observed and the value (11.1 ± 0.4 µg/ml) was only half of that measured with living bacteria.

Effect of P. fluorescens and LPS on the RMP of cerebellar granule neurons

The measures were performed on neurons exhibiting at least 2-3 adherent bacteria after 4 hours of incubation. The resting membrane potential of granule neurons exhibiting bacteria was significantly ($P < 0.001$) less negative (- 46.0 ± 4.7 mV, n=9) than in control neurons (- 63.7 ± 2 mV, n=12) (Fig. 3). The LPS extracted from *P. fluorescens* MF37 (200 ng/ml) provoked a membrane potential shift of the same range as that measured with the intact micro-organism (- 47.2 ± 4.8 mV, n=9) and this value was also significantly different ($P < 0.001$) from the control (n=9). In order to verify that the effect of the LPS was independent of artefacts the same measure was performed using the extraction buffer used to purify the endotoxin. Under these conditions, the resting membrane potential (-66.5 ± 2.4 mV, n=12) did not significantly differ from the control.

Invasive behaviour of P. fluorescens in cerebellar granule neurons

The microscopic acridine orange internalisation assay allowed us to visualise clearly stained bacteria in the cytoplasmic compartment of neurons (Fig. 4A). Counting of homologous fields showed that an average of 58% of granule neurons contained bacteria in their cytoplasm. This value is above the 5% accepted as the upper limit value to consider that a bacterium has an invasive behaviour [19]. The blank test performed for the gentamicin survival assay revealed that gentamicin 300µg/ml was capable of provoking a total destruction of *P. fluorescens* MF37 (10^6 CFU/ml) in 1 hour.

When cultured neurons were incubated with bacteria for 4 hours and then exposed for 1 h to gentamicin (300µg/ml), a significant number of viable bacteria were detected, suggesting that these micro-organisms were protected from gentamicin by the cytoplasmic membrane of the cells (Fig. 4 B). This population represented 32.6 ± 3.7 % of the total number of remaining bacteria associated with cultured neurons after a 4 h incubation and 3 rinsing steps. This percentage of invasive bacteria is in the same range as that obtained by the acridine orange internalisation assay.

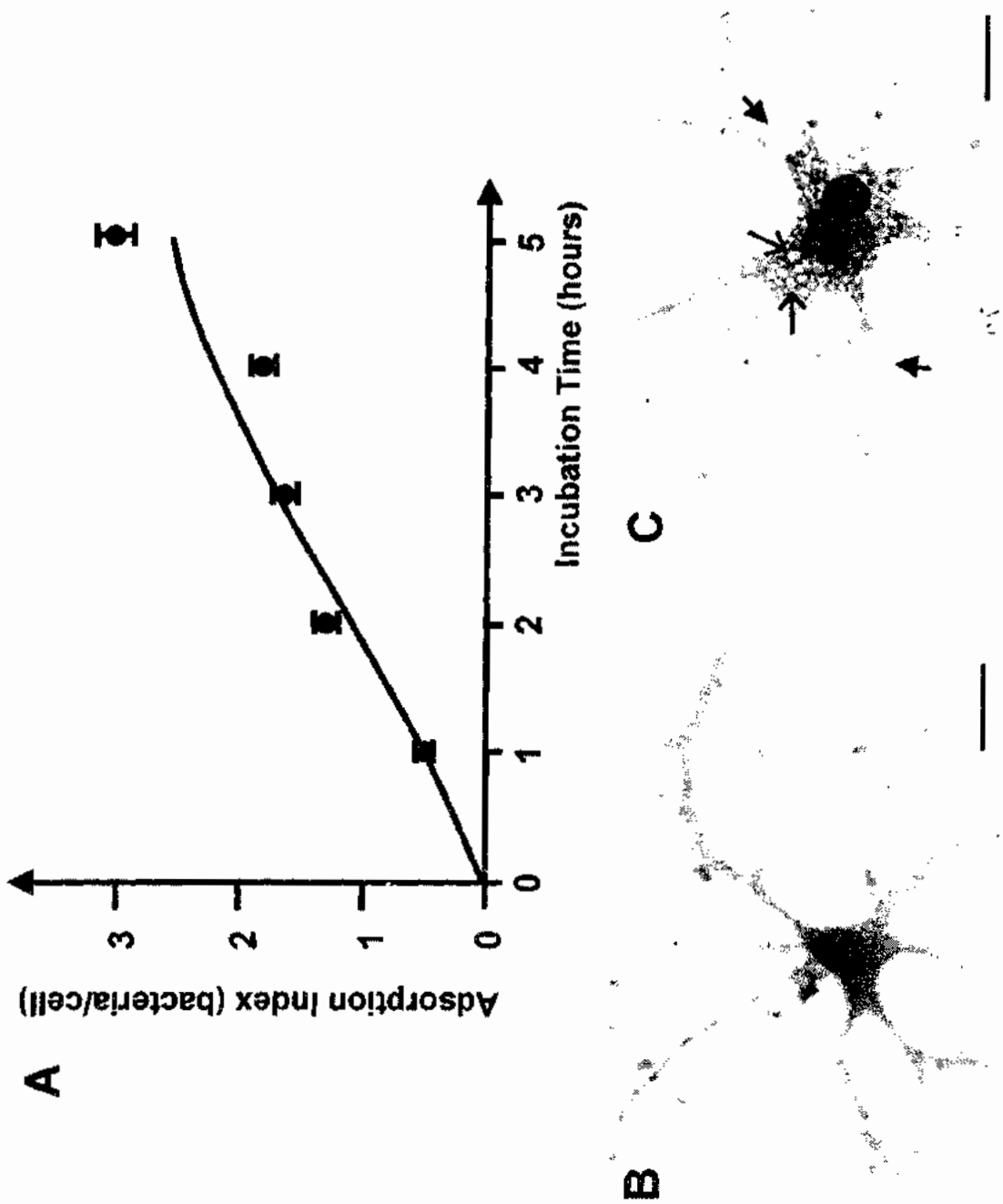


Fig. 1: Effects of *Pseudomonas fluorescens* MF37 (10^6 CFU/ml) on cerebellar granule neurons at day 7. (A) Time-course curve of the adherence of the bacteria. Each point corresponds to the mean number of adherent bacteria/neuron (± SEM) and was determined over a minimum of 50 neurons. (B-C) Photomicrographs of cultured cerebellar granule neurons in the absence (B) or presence (C) of bacteria after 5h exposure. Note that neurites have lost their cytoplasmic content (⟶) while the soma is markedly vacuolated (⟶).

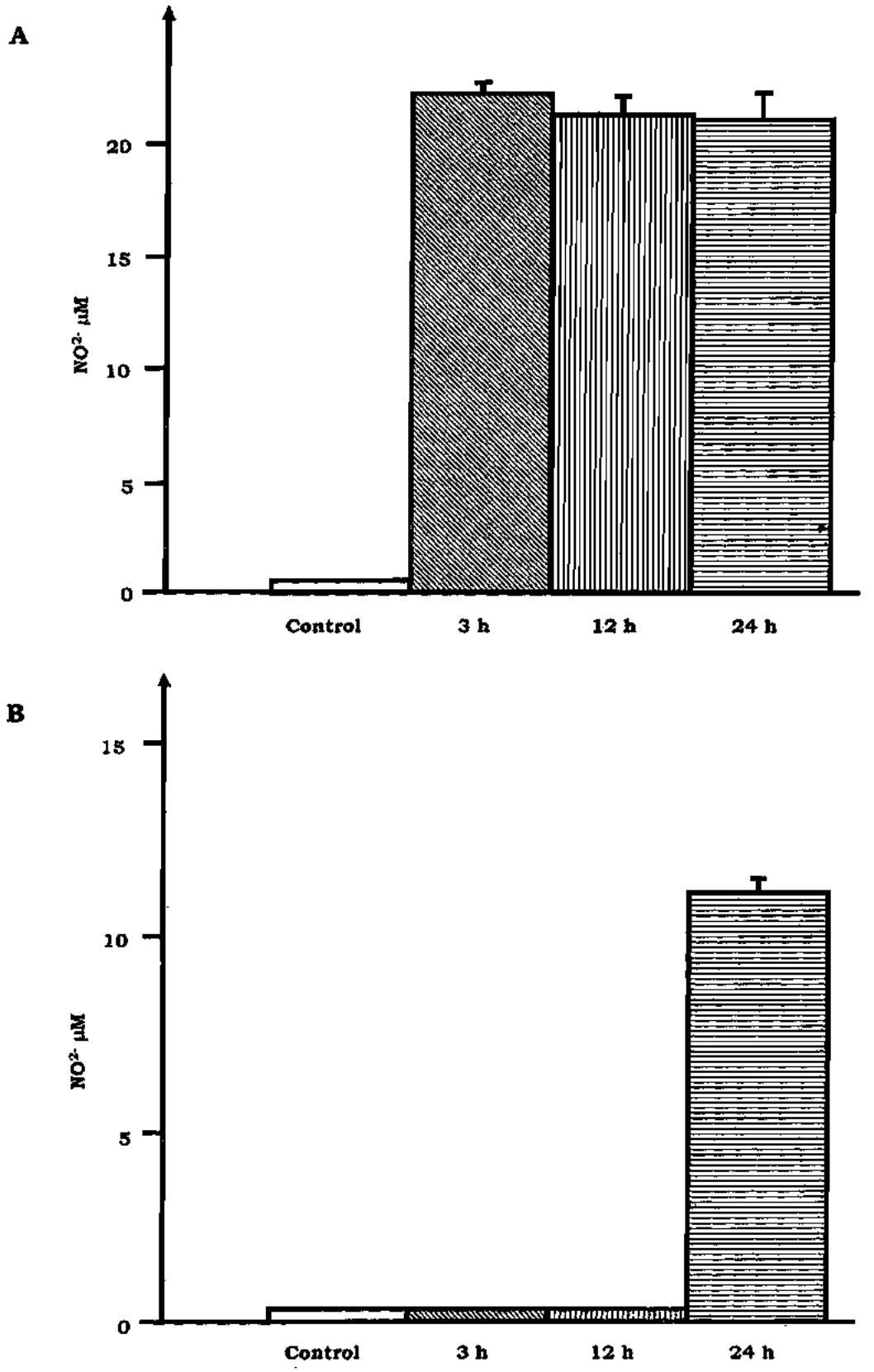


Fig. 2 Effects of *Pseudomonas florescens* and its LPS on NOS activity of cerebellar granule neurons. (A) Effects of the living bacteria (10^6 CFU/ml) on concentration of NO^2 measured in culture medium after 3, 12, and 24 hours of incubation. Control studies were performed to show absence of NOS activity in *P. florescens* MF 37 in the experimental conditions. (B) Effects of the LPS extracted from *P. florescens* (200ng/ml) on the concentration of NO^2 measured in culture medium after 3, 12 and 24 hours of incubation

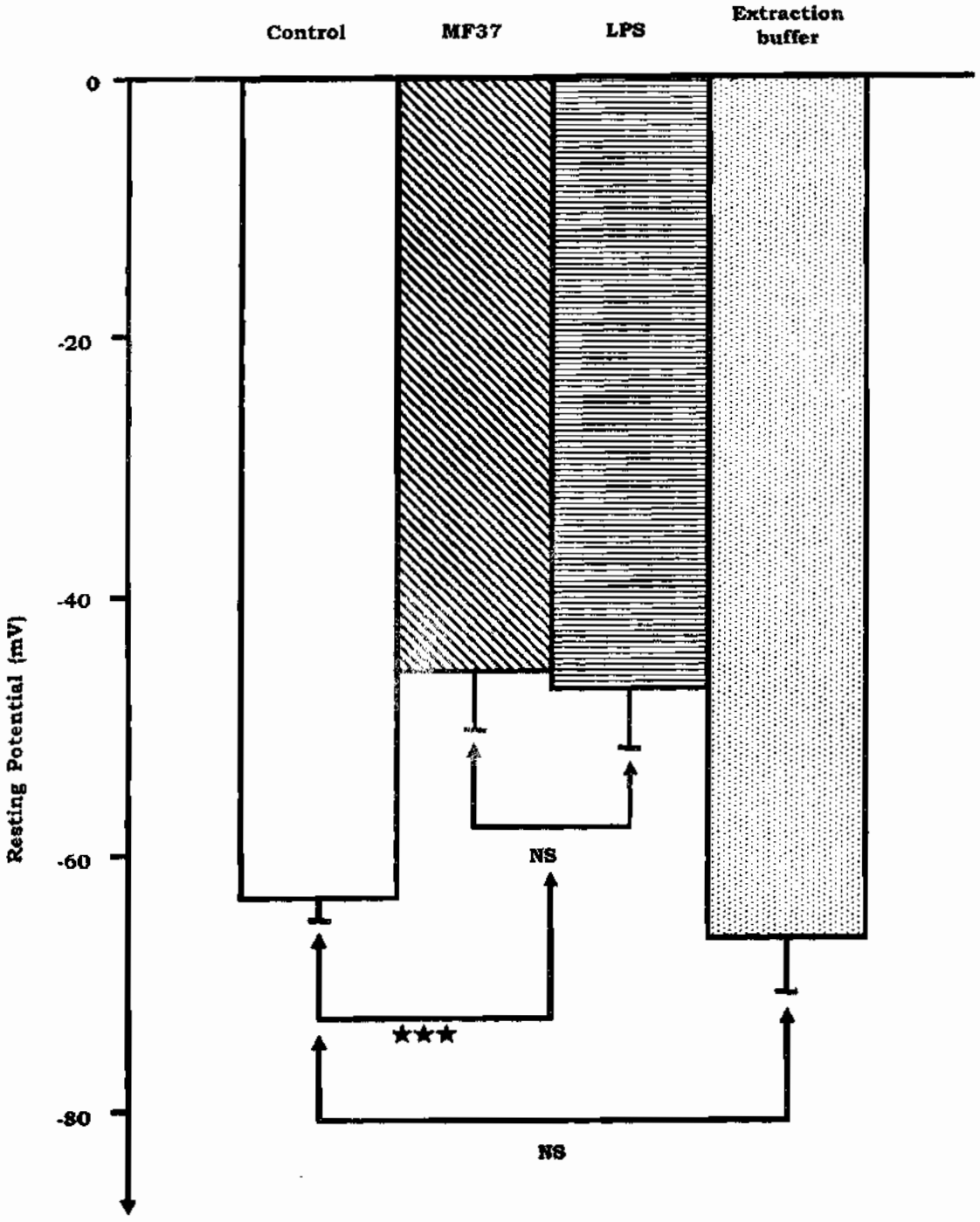
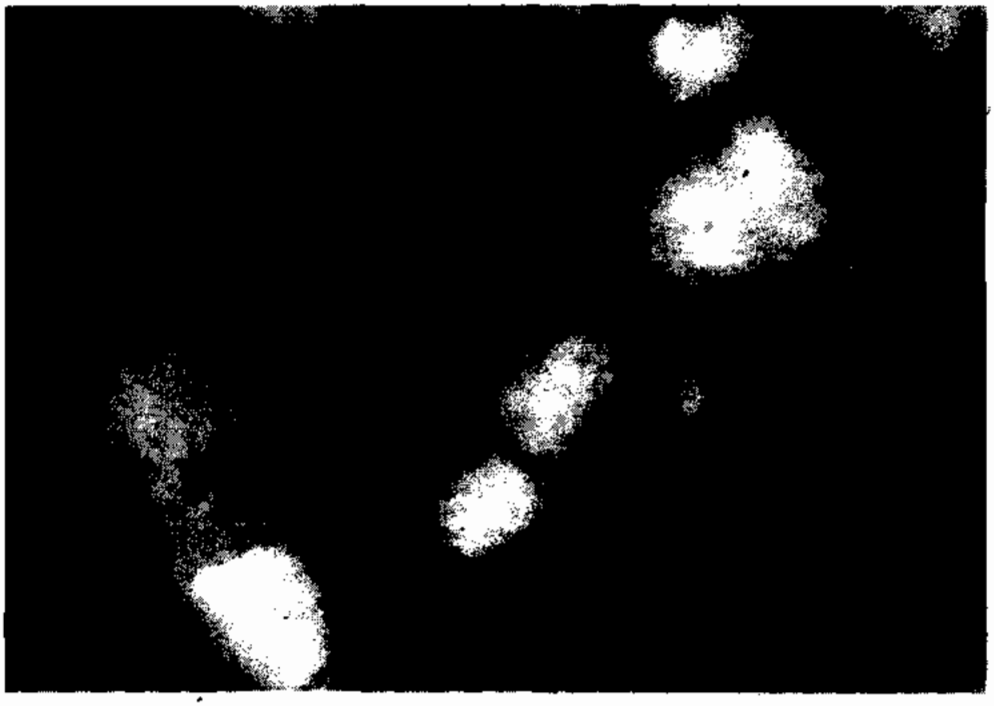


Fig. 3. Effects of *Pseudomonas florescens* MF 37 and its LPS on the resting membrane potential (RMP) of cultured cerebellar granule neurons at day 7. The histograms represent the level of the RMP in control neurons (Left bar), neurons incubated for 4 h with *P. fluorescens* 10^6 CFU/ml and exhibiting at least 1-2 adherent bacteria (Second bar), neurons incubated for 3 h with 200 ng/ml LPS (Third bar) or with the extraction buffer employed for the purification of LPS (Fourth bar). Each bar corresponds to the mean value (± SEM) of 9 measurements from 3 independent experiments.



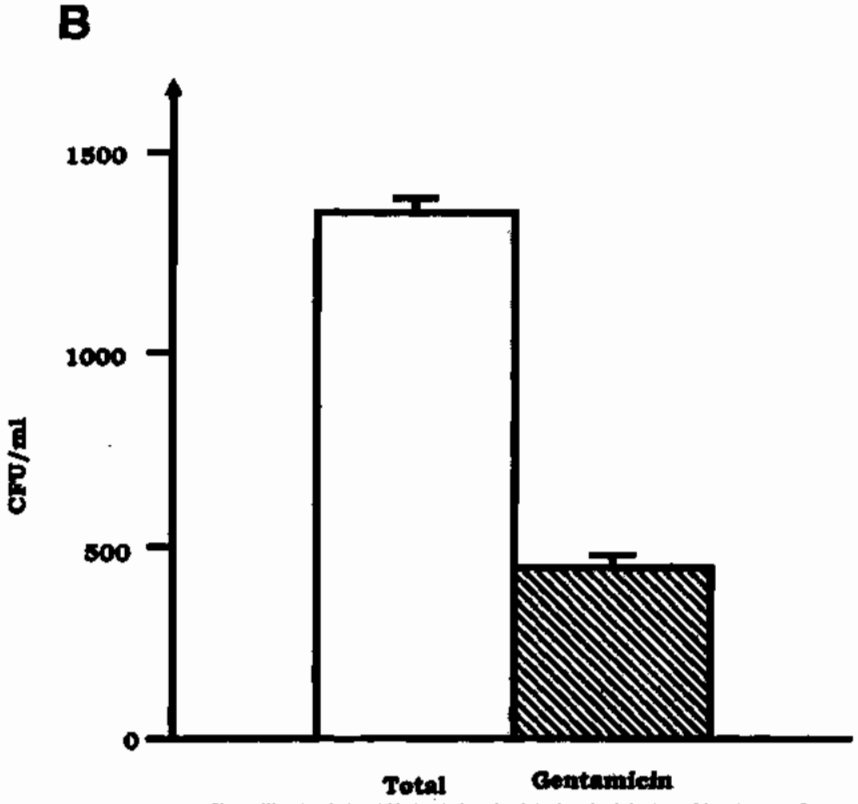


Fig. 4. Histochemical and biochemical study of the invasive behaviour of Pseudomonas fluorescens MF37 on cerebellar granule neurons. (A) Photomicrograph showing bacteria labelled by acridine orange in the cytoplasm of granule neurons. The counting of homologous fields showed that a mean of 58% of granule neurons contained labelled intracellular bacteria. (B) Gentamicin survival assay of P. fluorescens on cerebellar granule neurons. The left bar illustrates the total number of living bacteria (determined as CFU/ml) on or into granule neurons after 4 h of incubation. The right bar illustrates the number of living bacteria (determined as CFU/ml) present into granule neurons after 4 h of incubation and destruction of extracellular bacteria by gentamicin (300μg/ml). A control study was realized to verify that gentamicin (300μg/ml) induces a total destruction of non-protected P. fluorescens MF37 in the experimental conditions.

DISCUSSION

Since it is postulated that the virulence factors produced by *Pseudomonas* are essentially released in response to a cascade of events initiated by the binding of the bacterium to its host [1], a first step of our study was required to determine the kinetics of binding of *P. fluorescens* on cerebellar granule neurons, a cell type on which these bacteria have not been investigated before. The profile of the binding curve of *P. fluorescens* on cerebellar granule neurons was similar to that previously observed on cortical neurons [7]. In contrast, the binding index of *P. fluorescens* on cerebellar granule neurons was limited to a mean of 3 bacteria per cells whereas it reached 12 bacteria per cell in cortical neurons [7]

The heterogeneity of the cell types present in cortical neurons in primary culture is much higher than in cerebellar granule neurons cultures and the difference of binding index may be ascribed to the presence in the population of cortical neurons of cell lines presenting a very high affinity for bacteria. However, the limited variations of the statistical values in both studies is not in favour of this hypothesis and, since the size of cortical and cerebellar neurons in primary culture is in the same range, we can presume that the number of binding sites for bacteria in cerebellar neurons is lower than in cortical neurons. In spite of these differences, the morphological effects of *P. fluorescens* are similar in cerebellar and cortical neurons. Within 5 hours of incubation with the bacteria, cerebellar granule neurons presented vacuolar inclusions and nuclear aggregations typical of apoptosis [20]. Thus, cerebellar granule neurons appeared as a valuable representative model for the study of the time course effect of *P. fluorescens* and its LPS.

Until now, the effect of *P. fluorescens* on the formation of NO^{2-} in the culture medium of eukaryotic cells, which results from the activation of NOS, was only determined after 24 hours of exposure to the bacteria [13]. The present study revealed that the activation of NOS in granule neurons is very rapid since 3 hours incubation with *P. fluorescens* is sufficient to reach the maximum response. As clearly indicated by experiments of brain ischemia in animal models, the stimulation of NOS is an early event in the apoptotic process of neurons [21] and the observation of a rapid rise of the concentration of nitrites (NO^{2-}) following exposure of neurons to bacteria is in agreement with our previous observations [7].

A striking result was the delay in response of neurons to the LPS extracted from *P. fluorescens*. In addition, even after 24 h of incubation, when a rapid increase in the production of nitrites was observed, the level of NO^{2-} measured in the medium was only half of that obtained using the living bacteria. The LPS was used at a concentration (200ng/ml) corresponding to that potentially released by bacteria at the concentration employed in the present study (10^6 CFU/ml) [9]. Even though LPS probably plays a major role in the cytotoxic effect of *P. fluorescens* MF37, the present results suggest that at least another virulence factor is essential in the very early effects of the bacteria. In cerebellar granule neurons, two types of NOS have been identified, a constitutive type 1 NOS (nNOS), the activity

of which depends on calcium/calmodulin, and an inducible or type 2 NOS (iNOS) [22]. Rodrigo *et al* [23] have shown that an increase in the level of expression of iNOS can occur within 2 to 4 hours after stimulation. A concomitant activation of nNOS can also take place. However, a delay of 3 hours is short to provoke a maximal induction of iNOS, the massive accumulation of its metabolite (NO) and its spontaneous conversion into nitrites, as detected in the medium. Thus, it appears that the rise of NO observed with intact bacteria within 3 hours may be essentially ascribed to a direct activation of nNOS whereas the action of LPS, characterised by a long lag phase, could correspond to the activation of iNOS.

The activity of nNOS is controlled by transient increases of calcium resulting from the opening of voltage dependent calcium channels activated by membrane depolarisation [22]. The resting membrane potential of cerebellar granule neurons was studied using matured cells at 7 DIV since, as previously observed by Shibata *et al* 18], we observed that the resting membrane potential of the cells, initially less negative (- 41 ± 1.2 mV (n=12) at days 2-3), stabilised from day 7 to a mean value of - 63.7 ± 2 mV (n=9). The resting membrane potential of neurons was measured in cells exhibiting 2 to 3 adherent bacteria and in cells exposed for 3 hours to LPS. Living bacteria and LPS both induced a significant shift of the resting membrane potential to less negative values, - 46.0 ± 4.7 mV and - 47.2 ± 4.8 mV (n=9), respectively ($P < 0.001$). These results were not biased by compounds present in the extraction buffer used to purify LPS as demonstrated by control studies. The absence of difference between the electrophysiological effect of the living bacteria and its endotoxin demonstrates that LPS is the major virulence factor responsible for membrane depolarization in cerebellar granule neurons. The absence of significant difference between the effect of bacteria and LPS in these experiments also indicates that the rise of NO measured after 3 hours using living cells is independent of LPS and probably involves other virulence factor(s).

The membrane depolarization observed in the present study is in agreement with our previous works, showing that in cerebellar granule neurons, LPS provokes the reduction of two of the major voltage-dependent potassium currents [8]. It is known that in neurons depolarization triggers the activation of N-methyl-D-aspartic (NMDA) receptors coupled to calcium influx and to the activation of nNOS [24]. Prolonged activation of NMDA receptors leads to apoptosis [25]. Thus, the present results suggest that the bacterium and its LPS provoke the biosynthesis of NO by two pathways resulting in cell death.

The hypothesis that *P. fluorescens* rapidly activates nNOS presumes that the membrane depolarization activates local calcium influx. This presumption is supported by the morphological changes provoked by the bacteria. Calcium influx plays a central role in cytoskeletal rearrangements involved in cell binding and internalisation of bacteria [10]. In the present study, we demonstrate using two different and complementary approaches that, in addition to binding to cerebellar granule neurons, *P. fluorescens* MF37 exhibit an invasive behaviour that can be

observed within 4 hours of contact, before any effect of LPS on NOS. As indicated from the gentamicin assay, *P. fluorescens* MF37 survived through the internalisation process, but since *P. fluorescens* MF37 is a psychrotrophic strain, it is impossible to determine if the bacteria were able to grow, in the intracellular compartment.

Nonetheless, since they suggest that rapidly after binding of *P. fluorescens* to its target cell, local changes in calcium concentrations coupled to internalisation occur, these results support the hypothesis of an early stimulation of the calcium dependent nNOS. Further experiments should be performed to characterise the calcium channels involved in this process, but since the pharmacological agents used to selectively block ionic channels could also affect the bacterial physiology, this study deserves a complex electrophysiological approach. On the other hand, it is important to note that studies performed with *P. aeruginosa* have shown that in epithelial cells, invasion and cytotoxicity are independent and mutually exclusive events [17].

Thus, the situation of *P. fluorescens* MF37 appears more ambiguous since in addition to presenting a significant, and occasionally very active cytotoxic activity when grown at low temperature [13], this bacterium also exhibit an invasive behaviour. These results are in agreement with clinical observations showing that whereas *P. fluorescens* is generally a low virulence micro-organisms, some strains can behave as life threatening opportunistic pathogens [3].

Taken together, these results demonstrate that *P. fluorescens* can sequentially activate constitutive and inducible NOS expressed in cerebellar granule neurons. We have shown that LPS is essentially involved in a late induction of iNOS whereas other virulence factors are possibly responsible for the early effects of the bacterium and could control its invasive behaviour.

ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

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QUINOLONES RESISTANCE AND R-PLASMIDS OF SOME GRAM NEGATIVE ENTERIC BACILLI

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Out of the two hundred and sixty bacteria isolates from clinical specimens obtained from different body sites at the University College Hospital Ibadan; 166 belonged to the family of Enterobacteriaceae and Pseudomonaceae. The isolated gram-negative enteric bacilli consist of *Escherichia coli* (22), *Klebsiella species* (65), *Proteus species* (20), *Salmonella typhi* (2), *Pseudomonas aeruginosa* (39) and *Pseudomonas species* (18). Among the antimicrobial agents tested, high resistance was found with ofloxacin 44.0%, followed by pefloxacin 30.1% and ciprofloxacin 21.7%. Ciprofloxacin has the lowest MIC of 2 – 32 µg/ml while ofloxacin has the highest 64 µg/ml. Of the 166 strains, 44 were resistant to most of the antimicrobial agents tested. All the strains that were resistant to any antimicrobial agents were also resistant to ofloxacin. A total of 27 plasmids ranging in molecular sizes from 6.6kb to 17.4kb were extracted from the resistant strains and grouped into 5 plasmid profiles. Transformation experiment revealed that 59.2% of the resistant strains carried a common R-plasmid of size 10.7kb. Plasmid-mediated resistance to ciprofloxacin and pefloxacin was found. *Klebsiella species* harboured the highest number of R-plasmids with 8, followed by *Pseudomonas aeruginosa* with 4.

INTRODUCTION

The fluoroquinolones are a new class of synthetic antimicrobial agents which have greater activity against Gram positive and Gram-negative bacteria than the older quinolone analogue, nalidixic acid and oxolinic acid which are used for urinary infection and occasionally enteric infections (1, 2). In veterinary medicine, fluoroquinolones are used for treatment and metaphylaxis but not as growth promoters.

Quinolone resistance is increasing in clinical isolates and has reached a minimum inhibitory concentration (MIC) in *Escherichia coli* at 265 mg/ml for ciprofloxacin (3, 4, 5). The mechanism for this resistance involve chromosomal mutations that modify DNA gyrase or DNA topoisomerase IV the targets of quinolone action or results in decreased quinolone accumulation (6, 7, 8).

Multiple antibiotic resistance in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype (9-15). Transferable resistance to fluoroquinolones has been found in a clinical isolate of *K pneumoniae* in a broad host range plasmid (16).

Quinolone such as ciprofloxacin and ofloxacin have been introduced in to Nigeria while newer ones like pefloxacin and sparfloxacin are just being introduced in to Nigeria by some pharmaceutical companies under different trade names. These quinolones are used for various diseases ranging from urinary infections, enteric infections, septic wound, septicaemia etc. Some researchers have opined that susceptibility to quinolones may remain high in Nigeria as these drugs are expensive and beyond the reach of most individuals, but their use is increasing and resistance may become problematic in the years to come (17-20).

The recent incidence of emergence of resistance to the quinolones as reported by Oni *et al* (19) has prompted this present study. Thus this paper describes the antimicrobial susceptibility testing and plasmid screening of some gram-negative bacilli commonly isolated from clinical specimens in University College Hospital Ibadan, Nigeria.

MATERIALS AND METHODS

Bacteriology

Sixty-five strains of *Klebsiella spp.*, 22 strains of *Escherichia coli*, 20 strains of *Proteus spp.*, 18 strains of *Pseudomonas spp.*, 39 strains of *Pseudomonas aeruginosa* and 2 strains of *Salmonella typhi* isolated by standard procedures (21, 22) from 260 clinical specimens sent to the diagnostic laboratory of Medical Microbiology and Parasitology Laboratory of University College Hospital, Ibadan from May to December 2002 were studied.

Antimicrobial susceptibility testing

Antimicrobial disc susceptibility tests were carried out on the isolates using stokes disc diffusion technique (23) on freshly prepared Mueller-Hinton agar (Oxoid, England), and standardized by the method of National Committee for Clinical Laboratory Standard (24), using the following antibiotic discs; Pefloxacin 5μg (Peflotab), Ofloxacin 30 μg (Tarivid), Ciprofloxacin 5μg (Ciprotab), Ceftazidime 30 μg (Fortum), Ceftriaxone 30μg (Rocephine), Gentamicin 10μg (Abtek), Amoxicillin 25μg (Abtek), Augmentin 30μg (Abtek), Cotrimoxazole 30μg (Abtek). *Escherichia coli* (NCTC 10418) was used as control. Plates with antibiotic discs were incubated for 24 hours at 37oC and sensitivity pattern was compared with that of the control organism.

Minimum inhibitory concentration

The minimum inhibitory concentration (MICs) of ciprofloxacin, pefloxacin and ofloxacin for all the bacterial isolates was determined as described by Goldstein and Acar (3). Serial doubling dilution of the antimicrobials was made for the range of 0.0625 μg/ml to 256 μg/ml. Such stock diluted solution of ciprofloxacin, pefloxacin and ofloxacin was added to universal bottles containing 10ml volumes of nutrient broth to produce equivalent concentration of antibiotic required. Each dilution was inoculated with one drop (0.02ml) of the overnight broth culture containing 10^4 organisms/ ml of the test organisms using different sterile Pasteur pipettes. Five colonies of the species were then suspended in to 5 ml of Mueller Hinton broth and incubated overnight. The culture was then diluted 1 in 1000. The same proccdure was repeated for the control organisms *Escherichia coli* NCTC 109418. A tube of broth without antibiotic was also included to show the suitability of the broth for the growth of the organism and unseeded tube of broth with and without antibiotic to serve as controls of the sterility and clarity of the broth. All plates were incubated at 37°C for 18 hours.

Isolation and separation of plasmid DNA

Plasmid DNA was isolated, separated and stained as previously described (4). Plasmid profile groups were constructed by grouping strains possessing the same profile (containing the same number and molecular mass) or part of a profile constituting a core profile. Bacterial strains that carried the plasmid were regarded as constituting one plasmid group.

Genetic transfer

Transformation was done as described by Hanahan (25) using *Escherichia coli* K-12, HB101 (ara-14, galk2, hsd520, lacyl, leu, mtl01, pro A2, recA13, rpsl20, supE44, thii xyl-5) as recipient and plasmid pBR322 as the positive control. Co-transformation of resistant character was determined by testing all transformants against all antibiotics to which the donor strains was resistant, extracts from transformants were obtained as described above and subjected to agarose gel electrophoresis. Transformation was confirmed as positive only when resistant transformants were shown to contain a plasmid (s) of a size similar to that found in the original isolate.

Plasmid curing

The curing of the resistant plasmids of the clinical bacterial isolates was done as described by Vivyan *et al* (35).

RESULTS

The sources of the clinical bacterial isolates are shown in Table 1. The isolates were from various body sites.

Table 1: Sources of the bacterial isolates

Body sites	Bacterial isolates						Total
	E. coli	*K. spp*	*P. spp*	*Ps. aeruginosa*	*S. typhi*	*Ps. spp*	
Ear swab	0	8	9	19	0	7	43
Wound swab	13	23	9	13	1	6	65
Throat swab	1	3	1	0	0	0	5
Conjunctival swab	0	1	0	0	0	1	2
High vaginal swab	3	9	1	0	0	2	15
Endocervical swab	1	2	0	0	0	0	3
Sputum	0	7	0	6	0	1	14
Urine	3	8	0	1	0	0	12
Tracheal aspirate	1	2	0	0	0	1	4
Cerebrospinal fluid	0	2	0	0	1	0	3
Total	**22**	**65**	**20**	**39**	**2**	**18**	**166**

Table 3: MICs of a cumulative percentage of isolates with inocula of 10^5 CFU

Organisms (No. of Strains)	Antimicrobial Agents	MIC_{50}	MIC range (µg/ml)	MIC_{90}
Escherichia coli (22)	Ciprofloxacin	4	0.0313 – 64	8
	Pefloxacin	8	0.125 – 64	16
	Ofloxacin	8	0.125 – 128	32
Klebsiella spp (65)	Ciprofloxacin	4	0.0625 – 64	16
	Pefloxacin	8	0.25 – 128	32
	Ofloxacin	8	0.5 – 128	32
Proteus spp (20)	Ciprofloxacin	2	0.0313 – 32	4
	Pefloxacin	4	0.0625 – 64	8
	Ofloxacin	4	0.0625 – 64	16
Pseudomonas aeruginosa (39)	Ciprofloxacin	8	0.25 – 128	32
	Pefloxacin	16	0.5 – 128	64
	Ofloxacin	16	0.5 – 256	64
Salmonella typhi (2)	Ciprofloxacin	4	0.0313 – 32	4
	Pefloxacin	4	0.0625 – 32	8
	Ofloxacin	8	0.0625 – 32	16
Pseudomonas spp (18)	Ciprofloxacin	8	0.25 – 128	32
	Pefloxacin	16	0.5 – 128	64
	Ofloxacin	16	0.5 – 256	64

Key MIC_{50} Minimal inhibitory concentration for 50 percent of strains
MIC_{90} Minimal inhibitory concentration for 90 percent of strains
CFU = Colony Forming Unit

Of the 166 clinical bacterial strains isolated, 44 were resistant to most of the antimicrobial agents tested (Table not shown). The frequency of susceptibility to ceftazidime was the highest (83.1%) while the sensitivity to fluoroquinolones was, ciprofloxacin (78.3%), pefloxacin (69.9%) and ofloxacin (56.0%) (Table2).

Table 2: Antibiotic sensitivity pattern of some Gram negative enteric bacilli

Antibiotics	Number sensitive	% sensitive	% Resistant
Ciprofloxacin	130	78.3	21.7
Pefloxacin	116	69.9	30.1
Gentamicin	73	44.0	56.0
Amoxicillin	6	3.6	96.4
Augmentin	11	6.6	93.4
Cotrimoxazole	3	1.8	98.2
Ceftazidime	138	83.1	16.9
Ofloxacin	93	56.0	44.0
Ceftriaxone	124	74.7	25.3

All the strains that were resistant to any antimicrobial agents were also resistant to ofloxacin. The MIC expressed as MIC_{50}, MIC_{90} and range are shown in Table 3. Considering the Gram-negative bacilli, ciprofloxacin has the best sensitivity result, followed by pefloxacin and then ofloxacin. A total of 27 different plasmids with molecular mass ranging from 6.6kb to 17.4kb were observed in the antibiotic resistant strains. Plasmids were not detected in 17 of the resistant strains indicating that their resistance was probably chromosomally borne. Five different plasmid profile groups for the antibiotic resistant strains could be defined. The number of strain per plasmid profile group vary from 1-24 (Table 4).

Table 4: Plasmid profile groups of antibiotic resistant bacterial strains

Plasmid Profile	No. of strains	Molecular mass (kb) of plasmids
0	17	No plasmids
1	24	10.7
2	1	10.7, 6.6
3	1	10.7, 17.4
4	1	11.7

The most common antimicrobial resistance pattern was CipPefOfxGenAmxAugCotCazCro. This was followed in decreasing order of occurrence by the R-types resistance patterns; CipPefOfxGenAmxAugCotCazCro,PefOfxGenAmxAugCotCazCro,CipPefOfxGenAmxAugCot,OfxAmxAugCotGen,CipPefOfxGenAmxAugCotCro, and CipOfxGenAmxAugCotCazCro (Table 5).

Table 5: Antimicrobial resistance patterns of 44 clinical bacteria strains in relation to plasmid contents

Antimicrobial Resistance patterns	No	%	No with plasmids
Cip Pef Ofx Gen Amx Aug Cot Caz Cro	9	20.5	6
Pef Ofx Gen Amx Aug Cot Caz Cro	8	18.2	3
Cip Pef Ofx Gen Amx Aug Cot Caz Cro	1	2.3	1
Cip Pef Ofx Gen Amx Aug Cot Caz Cro	11	25.0	7
Cip Pef Ofx Gen Amx Aug Cot Caz Cro	4	9.1	4
Cip Pef Ofx Gen Amx Aug Cot Caz Cro	6	13.6	4
Ofx Amx Aug Cot Gen	5	11.4	2

Key: Cip = Ciprofloxacin, Pef = Pefloxacin, Ofx =Ofloxacin, Gen = Gentamicin, Amx = Amoxicillin, Aug = Augmentin, Cot = Cotrimoxazole, Caz = Ceftazidime, Cro =Ceftriaxone

Strains showing the resistance pattern, CipPefOfxGenAmxAugCotCazCro, harboured the highest number of plasmids while the lowest number was found in the single strain (*Klebsiella spp*) with the resistance pattern, CipPefOfxGenAmxAugCotCazCro.

Transformation experiment showed that 59.2% of the resistant strains that harboured plasmids were able to transfer their resistance plasmids to *E. coli* k-12 HB 101. Plasmid-determined resistance to ciprofloxacin and pefloxacin was found. It is noteworthy that all the R-plasmids isolated in this study have a common molecular size of 10.7Kb (Table 6).

Table 6: Characteristics of some of the clinical bacterial R-plasmids

Bacterial strain	Plasmids molecule size (kb)	Antibiotic gene transferred to *E. coli* Hb101	Transformant R-plasmid size (kb)
MmB8 (*K. spp*)	10.7	Pef	10.7
MmB10 (*Ps. aeruginosa*)	10.7	Cip	10.7
MmB11 (*K. spp*)	10.7	Cip Pef	10.7
MmB17 (*Ps. aeruginosa*)	10.7	Cip Pef	10.7
MmB30 (*K. spp*)	10.7	Cip Pef	10.7
MmB32 (*K. spp*)	10.7	Cip Pef	10.7
MmB36 (*K. spp*)	10.7	Pef	.0.7
MmB37 (*Pr. Spp*)	10.7	Cip Pef	10.7
MmB41 (*Ps. aeruginosa*)	6.6, 10.7	Pef	10.7
MmB42 (*K. spp*)	10.7	Cip Pef	10.7
MmB43 (*Ps. aeruginosa*)	10.7	Pef	10.7

It is also significant that strain MmB4, *Pseudomonas aeruginosa* was able to transfer its 10.7Kb R plasmid to *E. coli* HB101. All the strains harbouring R-plasmids were cured of their plasmids upon treatments with sodium dodecy sulphate (SDS), with resultant loss of their plasmid-associated properties. This indicates that the antibiotic resistant genes of the bacterial strains used in this study were plasmid borne.

DISCUSSION

Most of the Gram-negative bacilli especially *Klebsiella species* and *Pseudomonas species* are intrinsically resistant to most antibiotics, a situation which favour their continued existence in hospital environment (13, 17, 18, 20, 27). This fact greatly contributes to the high incidence of these agents among the patients. The comparative disc sensitivities shown in Table 2 is similar to that obtained by Oni *et al* (19) and Ozumba (20). The increasing resistance of enterobacteriaceae to fluoroquinolones: ciprofloxacin (21.7%), pefloxacin (30.1%) and ofloxacin (44.1%), is in agreement with the studies of Threfall *et al* (4), Nema *et al* (28), Gara *et al* (29). In this study *Pseudomonas aeruginosa* had 85% sensitivity to ciprofloxacin in contrast to the report of Odugbemi *et al* (18) which documented a 100% sensitivity of *Pseudomonas aeruginosa* to ciprofloxacin in 1994 in Lagos, Nigeria.

Most strains have MICs > 4 µg/ml with values as high as 64 µg/ml for ofloxacin. Comparatively ciprofloxacin has the lowest MIC of the fluoroquinolones used in this study. This is similar to the study of Threfall *et al* (4) in which high level resistance to ciprofloxacin in *Escherichia coli* with the MIC's range of 4-8 µg/ml. The antimicrobial resistance pattern revealed a total of seven patterns. This indicates the emergence of resistance to the quinolones in our environment. Also these patterns depict the occurrence of multiresistant strains. This is similar to that obtained by Zafar (30), Wallace *et al* (31) and Livermore *et al* (1). All the strains that were resistant to any antimicrobial agents were also resistant to ofloxacin.

Resistance to high levels of antibiotics has been ascribed in most instances to the presence of plasmids (9, 11,

12, 14, 15). Thus the presence of plasmids among the resistant strains is quite different from the report of Martinez-Martinez *et al* (16). The most common plasmids encountered were 10.7Kb in size. This is in agreement with the findings of Moller *et al* (32), Daini *et al* (9), Wang *et al* (33), and Ogunledun et al (13). 59.2% of the drug-resistant strains carried R-plasmids. Plasmid determined resistance to ciprofloxacin and pefloxacin was found. The emergence of R-plasmids in this study could be due to the indiscriminate and widespread use caused by the over-the counter availability of antibiotics as well as the higher exposure of people to enteric flora in places with poor sanitation (9, 10, 13, 18). A different plasmid profile could be seen for each of the 16 R-plasmids and plasmids of the same molecular weight could be found in different strains. Thus the plasmid profile of these strains was diverse in nature.

Plasmid profiling analysis distinguished more strains than the antimicrobial susceptibility patterns in agreement with the findings of Daini *et al* (9), Levy *et al* (12) and Senerwa *et al* (34). Plasmid profiling has been shown to be a good epidemiological tool in investigating epidemics or outbreaks of bacterial diseases (35, 36). The transformation experiment enabled us to detect non-self transmissible plasmids while curing of the resistant strains of the R-plasmids with SDS showed that their antimicrobial-resistant genes were plasmid-borne (9, 26).

The results of our study highlighted diverse plasmid profiles and widespread antimicrobial resistance patterns of some Gram negative enteric bacilli isolates from Nigeria and we hope that this information from this locality would be a useful baseline for further epidemiological studies.

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ORIGINAL ARTICLE

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PATTERN OF RESISTANCE TO VANCOMYCIN AND OTHER ANTIMICROBIAL AGENTS IN STAPHYLOCOCCAL ISOLATES IN A UNIVERSITY TEACHING HOSPITAL

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Multidrug resistance has been reported in clinical isolates of both coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* that are most often resistant to oxacillin/methicillin. Vancomycin, a glycopeptide is the drug of choice for infections caused by such multidrug resistant strains. This study determined the pattern of resistance to vancomycin and other antimicrobial agents in staphylococcal isolates from a University Teaching Hospital. Staphylococcal isolates from clinical specimens submitted to the diagnostic medical microbiology laboratory of the Ahmadu Bello University Teaching Hospital, Zaria (over a three-month period) were characterized using standard microbiological procedures and their susceptibility to vancomycin and other commonly used antimicrobial agents determined by Kirby-Bauer-NCCLS modified disc diffusion technique. A total of 56 of the 97 (57.7%) staphylococcal isolates characterized were resistant to vancomycin 30µg, showing a zone of inhibition less than 15mm. Most of these isolates were from urine (27.3%), wound (21.8%) and pleural aspirate (12.8%). The 56 staphylococcal isolates were made up of 75% (41/56) *Staphylococcus aureus* and 25% (14/56) *coagulase-negative staphylococci*. Majority of the isolates, 60.7% (34/56) produced β-lactamase enzyme. Resistance pattern to other antimicrobial agents was benzyl penicillin G (92.9%); tetracycline (69.6%); cefuroxime (60%); chloramphenicol (54.5%); oxacillin (49.1%); erythromycin (35.7%); gentamicin (25%) and ciprofloxacin (16.1%). Analysis of the multiple antibiotic resistance index (MARI) showed that majority (91.1%) were resistant to 3 to 7 of the other antimicrobial agents tested. No isolate was resistant to all the tested antimicrobial agents. A very high proportion of the staphylococcal isolates were resistant to vancomycin, a glycopeptide that is not commonly used in this environment. Ciprofloxacin and gentamicin appear to be the only agents that will be effective in treating infections by these isolates. The high proportion of isolates with MARI of 0.3 and above, suggest that the isolates originated from an environment where antibiotics are often used. There is need for constant, on-going antimicrobial resistance surveillance in important and commonly isolated clinically significant pathogens to form the basis for developing and implementing measures that will reduce the burden of antimicrobial resistance.

Keywords: vancomycin, methicillin resistance, *Staphylococcus aureus*, coagulase-negative staphylococci, antimicrobial agents

INTRODUCTION

Antimicrobial resistance is fast assuming an alarming proportion in many bacterial populations. The therapy of infectious diseases caused by bacteria resistant to multiple antimicrobial agents has emerged as one of the greatest challenges facing clinicians worldwide (1). The staphylococci present a special problem; with the coagulase-negative staphylococci (CoNS) associated with infections at the site of an indwelling catheter or cannula, cardiac and orthopaedic surgery involving the insertion of prosthetic devices and common cause of urinary tract infections (2). The coagulase-positive, *Staphylococcus aureus* is perhaps the pathogen of greatest concern because of its virulence (3), its ability to cause a diverse array of life threatening infections, and its capacity to adapt to different environmental conditions (3,4,5).

The first type of resistance to emerge in the staphylococci was the production of β-lactamase; enzymes that destroy the antibiotic by hydrolysing the β-lactam ring (6). Resistance to the β-lactamase stable penicillins (methicillin, oxacillin, nafcillin) is due to the production of altered cell-wall synthesis enzymes, the penicillin-binding proteins (PBPs) that are encoded on the *mec A* gene and function by preventing the binding of penicillin (6). Not many attempts have been made to document the prevalence of methicillin-resistant staphylococci in this environment (7, 8).

Vancomycin, a glycopeptide antibiotic was introduced into clinical practice in 1958 for the treatment of Gram-positive bacteria (4). The emergence and spread of vancomycin-resistant enterococci (VRE), the discovery that the genetic material responsible for high levels of vancomycin resistance in enterococci could be transferred *in vitro* to *S. aureus* was a great public health concern for many years (6,9). The development of very low levels of vancomycin resistance was first reported in CoNS in 1979 (10) and 1983 (11), while the first reported instance of vancomycin resistance in *S. aureus* occurred in Japan in 1996 (12). There have since been many other reported cases from other countries (10, 13-16). The mechanism of this resistance was observed to be distinct from that which mediates .vancomycin resistance in enterococci but appears to occur in a stepwise fashion as a result of long term, nearly constant exposure to vancomycin or glycopeptide (6) The glycopeptides (vancomycin or teicoplanin) are not used in this environment.

The purpose of this study was to determine the pattern of resistance in staphylococcal isolates from clinical specimens at the Ahmadu Bello University Teaching Hospital to vancomycin and other antimicrobial agents as a guide in assessing the need for the development of specific guidelines on the surveillance of this group of pathogens.

MATERIALS AND METHODS

Bacteriology

Staphylococcal isolates from all specimens submitted to the diagnostic medical microbiology laboratory of the Ahmadu Bello University Teaching Hospital, Zaria over a three-month period, were analyzed. The isolates were characterized using established microbiological methods, which included colonial morphology, Gram-stain characteristics, ability to produce enzyme peroxidase and coagulase to separate the *S. aureus* strains from the coagulase-negative staphylococci (17).

Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern of the isolates was determined using the Kirby-Bauer-NCCLS modified disc diffusion technique (18). All the strains were tested for their sensitivity to the following antibiotics; gentamicin 10µg, cefuroxime 30µg, chloramphenicol 30µg, erythromycin 15µg, tetracycline 30µg, vancomycin 30µg, benzyl penicillin G 10i u, oxacillin 1µg and ciprofloxacin 5µg (all from bio Mérieus sa 69280 Marcy l'Etoile-France). The zones of inhibition were recorded and the isolates classified as 'resistant'; 'intermediate' or 'sensitive' based on the interpretative chart updated according to the current NCCLS standards (18).

Test for β-lactamase production

Suspensions of the isolates were prepared by emulsifying bacterial colonies (from overnight nutrient agar culture) with sterile loops in 0.5ml of phosphate buffer solution containing 0.06mg/ml (10,000units/ml) of benzyl penicillin (penicillin G). As control, cell suspension of the standard typed culture of *Staphylococcus aureus* (ATCC 13709) was similarly set-up. They were incubated at room temperature for at least 1hour. Thereafter, 2 drops of freshly prepared 1% aqueous starch solution were added to each bacterial suspension and shaken. Then, 1 drop of iodine solution were added and allowed to stand for 10minutes at room temperature. β-lactamase producing organisms changed the colour of the reaction mixture from blue-black to colourless within the 10 minutes.

Determination of MAR index

The multiple antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant to by the total number of antibiotics tested (19, 20).

RESULTS

The distribution of the vancomycin-resistant staphylococcal isolates from various specimens is shown in Fig.1. A total of 56/97 (57.7%) of the staphylococcal isolates were resistant to vancomycin 30µg, showing a zone of inhibition less than 15mm (18). The vancomycin-resistant isolates were made up of *Staphylococcus aureus* 75% (41/56) and coagulase-negative staphylococci 25% (14/56). The resistance pattern of the vancomycin-resistant isolates to other antimicrobial agents is shown in Table 1.

Table 1: Antimicrobial Susceptibility of Vancomycin-Resistant Staphylococcal Isolates

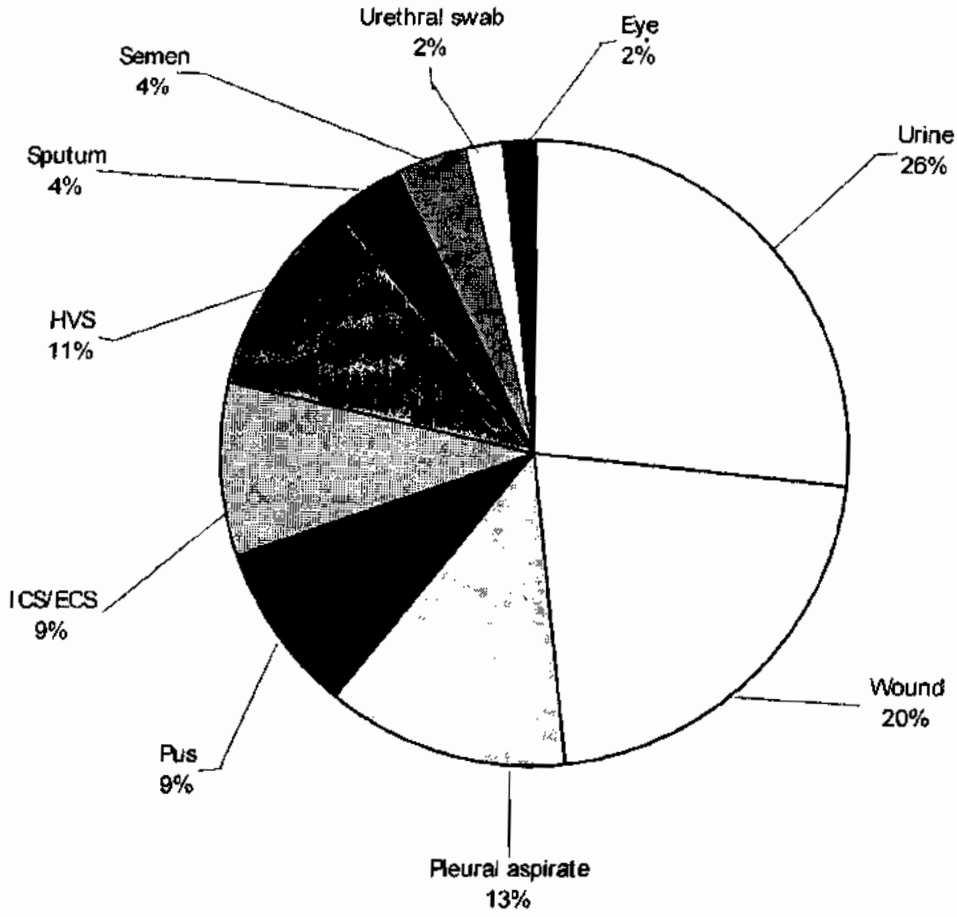
Antimicrobial agent	Disc Potency	% Resistant
Gentamicin	10µg	25
Cefuroxime	30µg	60
Chloramphenicol	30µg	54.5
Erythromycin	15µg	35.7
Tetracycline	30µg	69.6
Penicillin G	10i.u	92.9
Ciprofloxacin	5µg	16.1
Oxacillin	1µg	48.2

Only 27/56 (48.2%) of these vancomycin-resistant staphylococcal isolates were either oxacillin-resistant *Staphylococcus aureus* (ORSA) or oxacillin-resistant coagulase-negative staphylococcus (ORCoNS). Analysis of the multiple antibiotic resistance index (MARI) of the isolates shows that 91.1% were resistant to 3 or more antimicrobial agents (Table 2).

Table 2: Multiple Antibiotic Resistance Index of Vancomycin-Resistant Staphylococcal Isolates

MAR Index	No. of Isolates	Percentage
0.1	2	3.57
0.2	3	5.36
0.3	9	16.07
0.4	7	12.50
0.6	10	17.86
0.7	11	19.64
0.8	8	14.29
0.9	6	10.71

Fig. 1: Distribution of Vancomycin-Resistant Staphylococcal Isolates in Clinical Specimens



DISCUSSION

The level of vancomycin resistance in the staphylococcal isolates in this study was 57.7%. A previous study on the antibiogram and β-lactamase production of *Staphylococcus aureus* isolates from different clinical specimens (21) reported 100% sensitivity of the 73 isolates to vancomycin. Historically, the dramatic increase in the use of vancomycin in the treatment of infections caused by methicillin-resistant staphylococci (both coagulase-positive and coagulase-negative), *Clostridium difficile*, and the enterococcal infections preceded the emergence of vancomycin-resistant staphylococci internationally (5, 22). There is no such history of vancomycin use in this environment.

Vancomycin resistance can be difficult to detect in the clinical laboratory (4). Tenover *et al* (23) reported that the disc diffusion sensitivity testing using standard 30µg vancomycin disc frequently misclassify intermediately susceptible isolates as fully

susceptible. In a study (24), 75% of microbiology laboratories around the world actually misreported a glycopeptide-intermediate strain of *S. epidermidis* as susceptible based on the results of disc diffusion testing. The likelihood in this present study is the under detection of vancomycin resistance.

All strains of glycopeptide-resistant *Staphylococcus aureus* recovered to date are all oxacillin/methicillin resistant and were not clonal. Many of the patients had received vancomycin and had ORSA/MRSA infections (5, 6, 25). In this present study, only 48.2% of the isolates were either ORSA or ORCoNS. Since there was no perceived danger from ORSA or ORCoNS infections in this environment, isolates are not routinely tested for susceptibility to oxacillin/methicillin, therefore, there has been no need for the inclusion of vancomycin disc in sensitivity testing or on the hospital formulary and procurement by the hospital pharmacy.

Vancomycin resistant staphylococcal isolates in this study were resistant to 3 to 8 of the tested antimicrobial agents. This is consistent with the observation that all reported cases of glycopeptide-resistant *Staphylococcus aureus* (GRSA) cases have occurred in ORSA that are often resistant to wide variety of antibiotics (6). Most of the isolate's showed a reasonably high sensitivity to ciprofloxacin (83.9%), gentamicin (75%) and erythromycin (64.3%). Given the paucity of cases, there are no formal recommendations for the treatment of infections with coagulase-negative staphylococci with reduced susceptibility to vancomycin (4), but in one of the reported cases, the infection was successfully treated with erythromycin (26). Interestingly also, vancomycin intermediate *Staphylococcus aureus* (VISA) isolates in the United States were all sensitive to trimethoprim-sulfamethoxazole and tetracycline, while tobramycin, rifampicin, gentamicin, doxycycline are some of the drugs that have been successfully used clinically (4, 11, 27-30).

β-lactam antibiotics have also been used in the treatment of VISA cases, once in combination with aminoglycoside (12) and vancomycin (11). Only 48.2% of the vancomycin-resistant isolates were resistant to oxacillin 1μg. Although the specific role played by penicillin-binding proteins (PBPs) in vancomycin resistance remains unclear (4), studies have demonstrated that the MIC to oxacillin of some VISA isolates decreased as the vancomycin MIC goes up (31, 32). This supports the *in vitro* findings that the mutated penicillin-binding protein, PBP2a or PBP2' responsible for methicillin/oxacillin resistance is down regulated in vancomycin-resistant isolates (33). The high level of susceptibility of the vancomycin-resistant staphylococcal isolates to ciprofloxacin and gentamicin (two drugs frequently prescribed in this environment) may account for why infections by any of these isolates have not constituted a noticeable clinical problem.

There is however the need for more clinical data to assess the clinical significance of the vancomycin resistance encountered in this study and their attributable mortality and morbidity. There is also the need for consistent, on-going antimicrobial resistance surveillance for important and commonly isolated clinically significant pathogens like the MRSA, VISA, VRSA, and VRCoNS to form the basis for

developing and implementing measures that can reduce the burden of antimicrobial resistance and prevent a probable impending public health problem.

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OBSERVATIONS ON THE TOLERANCE OF YOUNG DOGS (PUPPIES) TO INFECTION WITH *TRYPANOSOMA CONGOLENSE*

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Studies were undertaken to assess the susceptibility of young local dogs to infection with *Trypanosoma congolense*. Six puppies (7 weeks old) were used for the study. Although the puppies became parasitaemic 6 to 7 days post infection, they were tolerant to infection as the parasitaemia remained low through out the first seven weeks of the eight week observation period. The packed cell volume (PCV) also only dropped slightly during the last four weeks attaining the value of 25.6 ± 3.8 ($p>0.05$) by the eighth week while the mean body weight continued to increase. Similarly, the mean daily body temperature did not differ significantly from those of un-infected control. The significance of trypanotolerance in Nigerian local dogs is discussed.

Keywords: local puppies, low parasitaemia, packed cell volume, *Trypanosoma congolense*, trypanotolerance, Nigerian

INTRODUCTION

Trypanotolerance has been described as the relative capacity of an animal to control the development of the trypanosome parasite and to limit their pathological effects, the most prominent of which is anaemia (1-3). Natural resistance to trypanosomes and trypanosomosis is genetically determined (4). The decreasing efficacy of available trypanocidal drugs and the difficulty of sustaining tsetse control have increased the imperative need to enhance trypanotolerance through selective breeding either within breeds or through cross breeding (1, 5).

In West Africa, shorter breeds of cattle, N'Dama and Muturu and West African Dwarf (WAD) sheep and goat (6) are known to be trypanotolerant while not much is known about the tolerance status of the different breeds of local dogs. Beside, the known epizootiological roles dogs' play in the spread of African trypanosomosis in animals (7), canine trypanosomosis, is a devastating disease resulting to anaemia, infertility, abortions and death if not treated (8). Identification of trypanotolerant trait in breeds of Nigerian local dogs may be an effective tool in the control of disease in dogs.

Pathogenic trypanosome species infective to dogs include *T. congolense, T. brucei* and *T. evansi* (9). Dogs are also known to be readily infected by human infective *T. gambiense* (7) and *T. rhodesiense* (9). The disease caused by *T. congolense* may be both severe and fatal in dogs (8). In this study, we report clinical manifestation of trypanotolerance in young Nigerian local puppies infected with *T. congolense*.

MATERIALS AND METHODS

Experimental animals

A total of six (6) local puppies of 7 weeks old made up of 2 males and 4 females weighing 2.0 to 3.2kg body weight (BW) were used. All of the six dogs were both welped by the same mother at the Nigerian Institute for Trypanosomiasis Research quarters in Kaduna. The bitch was a local dog and

mounted by other local dogs within the area. The puppies were first of all acclimatised in their kennels at the institute laboratories for one week before use. During this period, they were de-wormed with Piperazine citrate, Dicestal(R) and Dinitrophenol against roundworms, tapeworms and hookworms respectively. They were regularly deticked with Diazinon, Diazintol, (R) at 2weeks intervals. The diet was made of milk, beans, rice, yams, vegetables, soybeans and meat occasionally. Water was provided *ad libitum*.

Trypanosome parasites

Parasites used were *T. congolense* (NITR/Federe) isolated from cattle. and cryopreserved in liquid nitrogen from where they were later sub-passaged once, into albino rats before use.

Experimental design and sample analysis

Four (4) of the puppies, Nos. 01, 02, 04 and 05, were randomly selected and infected with 1.0 x 10^6 of the parasites subcutaneously. The remaining 2 puppies; Nos. 03 and 06 served as uninfected control. Wet blood film taken daily from the ear vein of the infected puppies was used for estimation of parasitaemia under the x40 objective of the light microscope. 100 microscopic fields were examined before the result was declared negative.

Blood for estimation of Packed Cell Volume (PCV) and other haematological values was obtained through venipunctures of the femoral vein using 21 gauge hypodermic needle and 5mls syringes. Capillary tubes were 3/4 filled with the whole blood, sealed one end with plasticine and centrifuged for 5 minutes in a microhaematocrit centrifuge at 12,000G. The PCV was read off the haematocrit reader (10). Whereas daily rectal temperatures were obtained with the help of the clinical thermometer, body weight of the puppies was obtained weekly using a balance (Henson(R) Gallenkamp, England). The data was analysed using student t-test.

RESULTS

The young puppies became parasitaemic with *T. congolense* 6 to 7 days post infection (PI). However, parasitaemia remained low with mean log equivalent value (LEV) of 1.07 $\pm$0.57 which lasted for seven weeks but only increased to 3.36 $\pm$ 0.32 ($P<0.05$) by week 8 PI (Fig. 1).

The Packed Cell Volume of infected dogs continued to increase from the mean pre-infection value of 27.85 $\pm$ 0.21% for the first 4 weeks of infection attaining maximum value of 31.00$\pm$1.68% by week 3 PI and later declined in the last 4 weeks to the value of 25.6 $\pm$3.81(%) by week 8 PI ($P>0.05$; Fig 2), while the values of uninfected control continued to rise. The over all changes in the mean PCV values of control and infected dogs did not differ from each other (Table 1).

The mean average body weight of the *T. congolense* infected dogs was not adversely affected as it continued to increase form pre-infection value of 2.6 $\pm$0.14 kg attaining the maximum weight of 4.8$\pm$ 0.7kg by week 8PI (Fig 3), when the experiment ended. The overall changes in the mean daily body temperature of infected puppies also did not differ from those of control puppies ($P>0.05$, Table1).

Table 1: Summary of overall changes in the mean value of packed cell volume, body temperature and body weight of control and *T. congolense* infected puppies.

	Control	Infected	
		Pre-infection	Post-infection
Packed Cell volume (%)	29.2±2.42	27.85±0.21	28.13±1.15
Body temperature (°C)	38.01±1.09	38.5±0.26	38.71±0.43
Body weight (Kg)	3.63±0.67	2.60±0.14	3.79±0.75

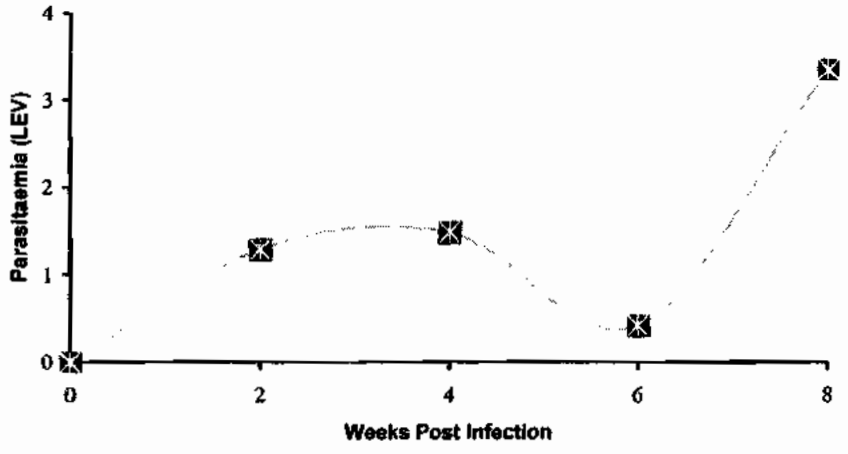

Fig 1: Mean Parasitaemia (LEV) of puppies infected with *T. congolense*

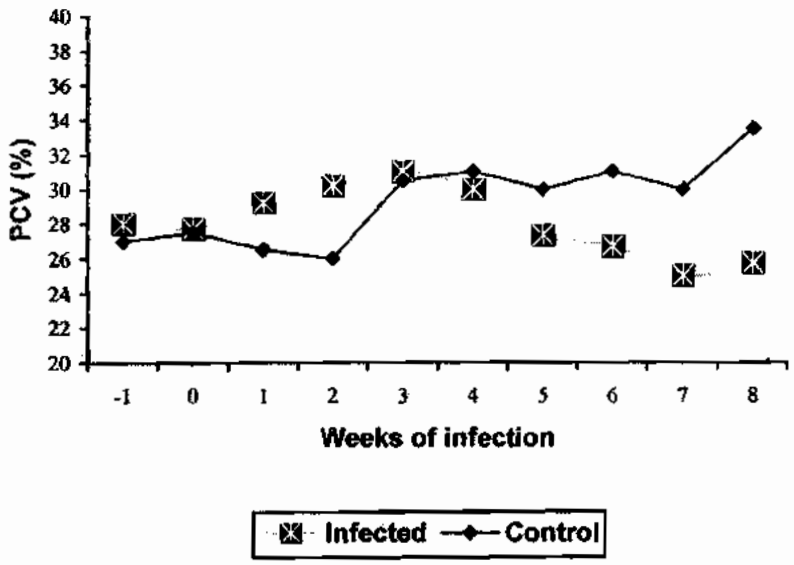

Fig 2: Packed Cell Volume (%) of *T. congolense* - infected and control puppies

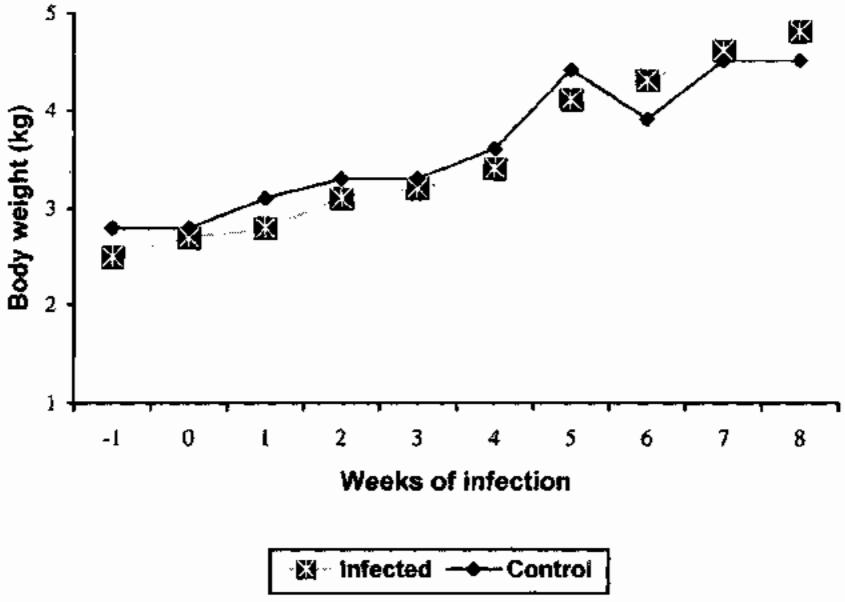

Fig 3: Changes in the Mean body weight (Kg) of *T. congolense* - infected and control puppies

DISCUSSION

Innate ability of trypanosome infected animals to control anaemia and development of parasitaemia have been identified as indicators of trypanotolerance (2). The parasitaemia of *T. congolense* infected puppies observed here was relatively low for seven weeks and increased only by the eighth week. This is at variance with the fulminating parasitaemia observed in *T. congolense* infected trypanosusceptible mice (11) and Nigerian Yankassa sheep (12) in which the animals died within one week and in less than 4 weeks PI respectively. The ability of the young dogs to control parasitaemia within the 8 weeks observation period indicates genetically determined tolerance to *T. congolense*.

Similarly, the Packed Cell Volumes of the infected puppies did not fall significantly throughout the period indicating innate ability to control development of anaemia (1, 2). These observations compare well with those of *T. congolense* infection in trypanotolerant sheep and goats (13) in which infected animals exhibited chronic anaemia and were able to control parasitaemia over a long period. Omotainse (14) however reported susceptibility of adult dogs to *T. brucei* infection resulting to death of some of the dogs 5 to 42 days post infection after developing low PCV.

Trypanotolerance is a genetically determined complex mechanism involving factors which are not yet well known. Naessens *et al* (3) reported two mechanisms involved in natural resistance to African trypanosomosis in cattle in West Africa,

namely; an innate mechanism that controls parasite growth, and another involving haemopoetic system that is able to limit anaemia. The low parasitaemia observed in *T. congolense* infected puppies may thus not be unconnected with ability of the dogs to control parasite development due to trypanolytic factors in the serum of infected dogs as has been demonstrated by Wang *et al* (15) in *T. brucei* and *T. congolense* infected cape buffalos. This has been shown to involve two factors; namely, complement-dependent and clone specific lytic activity and, complement-independent trypanocidal activity that are not restricted to trypanosome clones and species (15).

Similar anti-trypanosomal activity was demonstrated in the sera obtained from Cape buffalo, giraffe and greater Kudu resulting to inhibition of replication of *T. brucei* (16). Also, serum xanthene oxidase, serum catalase and trypanosome specific immune responses have been reported to play roles in regulation of the level of trypanosome parasitaemia in trypanotolerant Cape buffalo (17). Logan-Henfrey *et al* (18) reported that the bone marrow response is a key determinant factor of trypanotolerance in cattle as it determines the animal's capacity for haematopoietic cell regeneration and control of anaemia. This was supported by light and electron microscopic studies of sequential biopsies of bone marrow which showed key differences between trypanotolerant N'dama and trypanosusceptible Boran cattle. Such mechanism may also have been responsible for the low anaemia in *the T. congolense* infected puppies.

Almost normal Packed Cell Volume, progressive increase in body weight and normal body temperature observed in the infected young dogs confirmed the limited pathological effect of *T. congolense* on the puppies. Whereas further studies may be needed to confirm the trypanotolerant traits in Nigerian local dogs, the ability of the *T. congolense* infected puppies to resist parasitaemia and development of anaemia are indicative of trypanotolerance in the local puppies.

Trypanotolerance in local dogs may serve as an important measure against the current upsurge in cases of canine trypanosomosis in the country and limit mortality of exotic breeds of dogs through crossbreeding with the tolerant breeds.

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INFLUENCE OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ON THE SURVIVAL OF HIV-INFECTED PATIENTS: PART REPORT OF THE ILORIN TRIAL CENTER

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This report is part of the ongoing highly active antiretroviral therapy (HAART) trial, 167 patients were enlisted, but current analysis was restricted to 107 patients that were about a year old on the programme. The baseline weight, CD4+ cell count and serum albumin of 59 males and 48 females age 15-60 years, were compared with values at 12 months of administration of HAART. Patients mean weight, CD4+ count and serum albumin increased significantly (p-value < 0.05) by 9.7kg, 127.4/uL and 9.1g/L over the enrolment values. Side effects of antiretroviral (ARV) drugs were mild and included; rashes 19.6%, jaundice 7.5% and reactive arthritis 3.5%. Fifty-eight patients (59%) were alive by the end of 1 year, 33 (30%) had died and 11 (10.8%) were lost to follow-up. The risk of death increased 3 times when baseline CD4+ count was less than 116.8/uL (RR= 3.36, 95%CI=1.86- 6.06, P-value = 0.000048). TB/HIV co-infection raised the chance of death twice (RR= 2.33, 95%CI=1.31-4.15, P- value=0.005). In conclusion, the use of triple-drug combination of HAART has led to improved CD4+ cell count and resolution of clinical symptoms in HIV/AIDS patients. These resulted in increased survival.

Keywords: HAART, AIDS, CD4+ cells count and survival.

INTRODUCTION

The scourge of HIV/AIDS is progressing and causing devastation to lives and health care system worldwide. Most people infected with this disease live in resource poor countries, especially Sub Saharan Africa. Since the discovery of ARV drugs, there has been tremendous amelioration in the previously fatal clinical course of AIDS, these reflected in improved survival, decreased frequency and severity of opportunistic infection and improved quality of life (1).

However, despite the availability of these drugs and the increasing awareness of the immense benefit from it use, less than 5% of those who require treatment with these drugs have access to it (2). It has been estimated that in the year 2001 half of the 230,000 people receiving ARV in the developing world live in Brazil (2), while in the whole of Africa, the continent that is hardest hit by the HIV/AIDS pandemic, less than 50,000 people are receiving ARV therapy (2). This is why the Government of Nigeria should be commended on the recent provision of access to ARV drugs at a highly subsidized rate to the ever-increasing number of HIV/AIDS patients in the country.

As at 2003 there were about halve a million people living with AIDS in Nigeria, giving a national prevalence rate of 5.8% (3). Only 10,000 of this number have access to antiretroviral drugs (3). Government can be encouraged further to make continuous supply of these drugs one of her priorities among other contending health problems, if local evidences on the effectiveness or otherwise of these drugs are seen in the end users. Hence, we chose to document, using the experience of the ARV drug trial programme in our center, the influence of

HAART drugs on the survival of HIV-infected patients.

MATERIALS AND METHODS

University of Ilorin Teaching Hospital is one of the pilot clinical trial centers for the ARV drugs in the country, utilizing triple-drug combinations (one non-nucleoside; nevirapine and two nucleoside reverse transcriptase inhibitors; starvudine and lamivudine. The programme is at the concluding stage of its phase 2.

Adults Nigerian diagnosed by 2 different serological methods to be HIV positive, are eligible for inclusion in the programme. Patients referred from other health facilities were retested in our center before receiving ARV drugs. Cohorts of those who were about a year old on the programme were evaluated. Their records were analyzed for demographic information like age and sex. The clinical details, laboratory profiles and side effects of the medications were noted. Patients serial weights, $CD4^+$ cell counts and serum albumin were compared at enrolment and at 12 months of HAART administration using three set points: (i) clinical and immunological improvement on medication, (ii) death during the course of treatment, either in the hospital or at home, but reported by the relatives and (iii) lost to follow-up, when final outcome was not known.

Statistical method

Response to ARV drugs was determined clinically and immunologically by linear rate of increase in weight, serum albumin and $CD4^+$ cells count over the enrolment values. Paired-samples T- test was then employed to compare the means of each of these variables. Difference between the means was considered significant when p-value was less than 0.05. Risk of death was estimated by Chi-square analysis.

RESULTS

One hundred and seven patients, 59 (55%) males and 48 (45%) females, aged 19-60 years with a mean age of 36.4 years were seen during this period (Table1).

Table 1: Demographic and clinical variables of patients on antiretroviral drugs

Sex	Frequency	Percent
M	59	55
F	48	45
Age group		
15-24	11	10.3
25-34	35	32.7
35-44	39	36.4
45-54	14	13.2
≥55	8	7.4
Clinical features		
Fever	76	71
Diarrhea	69	64.5
Weight loss	67	62.6
Oral thrush	64	59.8
Tuberculosis	41	38.3
Rashes	31	29
Pneumonia	14	13.1
Clinical severity		
Stage 1	5	4.7
Stage 2	9	8.4
Stage 3	27	25.2
Stage 4	66	61.7

About 70% of these cases were in the 25-44 years age group. The most frequent clinical features were; fever 71%, diarrhea 64.5%, weight loss 62.6%, oral thrush 59.8% and rashes 29%. Tuberculosis occurred in 38.3% of the patients and pneumonia in another 13.2%. Over 85% of the patients fell within stages 3 and 4 of the WHO clinical staging system for HIV infection and disease.

The mean value of $CD4^+$ at enrolment was 194.9/ul; with a range of 40/ul -540/ul the lowest value occurred in 2 cases of TB. The mean serum albumin was

28.8g/L (range 15– 43g/L). There was statistically significant increase in all the monitoring variables (P-value < 0.0 5). Mean weight increased by 9.7kg, from enrolment value of 48.24kg to 57.9kg during the 12 months period (Table 2), while the mean value of CD4+ cell count increased by 127.4/uL; from enrolment value of 194.9/uL to 322.3/uL. The weight was moderately correlated with the increase in CD4+ count (correlation coefficient 0.58 (0.41-0.54). Mean values of albumin and Packed Cell Volume increased by 9.1g/L and 8.8% from baseline values of 28.7g/L to 37.8g/L and 24.1% to 32.9% respectively (Table 3). Increase in serum albumin was only mildly correlated with CD4+ change (correlation coefficient 0.42 (0.08-0.39).

Adverse drug reactions occurred in 29 patients (27%) on ARV drugs. Twenty-one (19.6%) of these were rashes, '8(7.5%) had jaundice and 4(3.5%) had reactive polyarthritis. All resolved spontaneously. Fifty-eight (59%) patients were alive and receiving care, while 11 (10.3%) were lost to follow-up and 5 patients (4.7%) refused ARV drugs because of the belief that AIDS is not curable. Thirty-three patients (30.8%) died; their mean enrolment CD4+ cell count was 116.8/uL. Twenty-one (64%) of the deceased had CD4+ count below this value and only 11 out of the total survivals had CD4+ count below 116.8/uL. The relative risk of death increased more than 3 folds when the CD4+ count was less than 116.8/uL (RR= 3.36, 95% CI =1.86 – 6.06, and p-value = 0.000048) (Table 4).

Table 2: Changes in variables used to monitor response to HAARTS therapy

Variable	Mean at inception	Mean at 9 months	Difference in mean	Standard error	95% CI difference	P-value
Weight	48.2	57.9	9.7	0.64	-10.9 to -8.4	0.000
CD4+	194.9	322.3	127.4	13.2	-153 to -100.9	0.000
Serum albumin	28.7	37.8	9.1	0.8	-10.6 to -7.4	0.001
PCV	24.1	32.9	8.8	0.72	-10.2 to -7.3	0.008

Table 3: Adverse effects of HAART and outcome of the pilot study

Variable	Frequency	Percent
Drug reaction		
Rashes	21	63.6
Jaundice	8	24.3
Arthralgia	4	12.1
Outcome		
Alive	58	54.2
Dead	33	30.8
Lost to follow-up	11	10.3
Refused treatment	5	4.7

Table 4: Factors responsible for death in HIV/AIDS patients on ARVS drugs

Variables	Dead	Alive	RR	95% CI	p-value
CD4 <116.8/uL	21	12	3.3	1.86-6.06	0.00005
PTB/HIV co-infection	21	18	2.3	1.31-4.156	0.005
PTB/CD4<116.8/uL	16	3	3.4	1.54-7.38	0.0007

Forty-one patients (38.3%) had PTB/HIV co infection, 18(44%) were still on ARV and anti-TB drugs, 21 (51.2%) died and 2(4.8%) were lost to follow-up. The risk of death in patients with co-infection was twice that of those with HIV infection only (RR= 2.33, 95%CI=1.31-4.15, p-value=0.005), while the risk of death was more than 3 folds in those with TB/HIV infection and $CD4^+$ less than 116.8/uL (RR=3.37, 95% CI=1.54-7.38). Of the 21 patients who died of TB/HIV co-infection, 16 had $CD4^+$ count less than 116.8/uL while only 3 of those who are alive and on treatment for both condition had count less than 116.8/uL.

DISCUSSION

The mean age of the HIV/AIDS patients was 36.4years with a slight male preponderance, M: F= 1.2:1. Over 70% of them were in the 25-44 years age group. The group that is prone to high-risk sexual behaviour such as multiple sexual partners and unprotected heterosexual intercourse. An individual living with HIV virus could develop AIDS from continuous replication of the virus, which will target the $CD4^+$ lymphocytes and other immune mediating cells of the body thereby decreasing the immune capability of such individual (4).

Knowledge of the life cycle of the HIV virus has led to the development of ARV drugs that also target the reverse transcriptase and the protease enzymes of the virus (4). Therapeutic trial of a triple-drug combination of ARV in our center showed disappearance or improvement of the enrolment symptoms and signs with associated significant changes in the clinical and immunological status of the patients. This experience was shared with similar trial programme of combination ARV therapy earlier conducted within and outside the country (5-8).

In this report, the mean weight of patients at enrolment increased by 9.7kg during the 12 months of ARV therapy and the mean baseline $CD4^+$ cells count increased by 127.4/uL over the same period of time. Serum albumin and PCV also increased by 9.1kg and 8.8% over their baseline values. Resolution of clinical symptoms that followed improvement in the patients' immunological status was reflected in the correlation coefficient of about 60% between the changes in the baseline $CD4^+$ cell count and the observed weight gained. Increase in $CD4^+$ cell count has been known to follow administration of HAART (9). This increase was even more in those receiving protease inhibitor-containing regimen (10) or better still showed a decrease in the viral load upon commencing ARV drugs (11).

In Nigeria, cost is an important limiting factor against the inclusion of protease inhibitors in the current combination of ARV regimen. The same could be said of provision of facilities for accessing and monitoring patients' viral load while on ARV therapy. The observed effectiveness of HAART in delaying the clinical progression of AIDS was not without some drawbacks. Thirty-three patients (30.8%) developed one form of reaction or the other; majority of these (63.6%) were rashes that resolved without intervention. Hepatic reactions occurred in 8 patients (24.2%). Medications were stopped momentarily in two of them. Four other patients (12.1%) developed reactive polyarthritis; it was effusive in two of them. They all responded to NSAID.

Outcome of this trial revealed that by the 12th month of the programme over 50% of the patients were alive and receiving maintenance care, while 11 patients (10.8%) were lost to follow-up and 5 patients (4.7%) refused administration of ARV drugs on the ground of lack of hope for survival. Thirty-three patients (30.8%) died, TB contributed to death in 21 (64%) of them, raising the risk of death more than twice. The risk of death was also raised by a low $CD4^+$ count, especially when it was lower than 116.8/uL. The risk of death was increased more than three times in those with count less than this. Combination of HIV/TB co-infection and $CD4^+$ count of less than 116.8/uL did not raise the risk of death more than that of a low $CD4^+$ counts alone. This probably suggests that baseline $CD4^+$ count is a very strong predictor of survival or death in AIDS patients. Some studies had earlier confirmed this observation (12, 13).

In the industrialized nations, much lower count of less than 72 cells/uL was associated with poorer outcome (14). Disparity in the predictive value of $CD4^+$ count of this magnitude could be a reflection of the advance level of care in terms of potent ARV drugs and prophylaxis against AIDS-related opportunistic conditions that are available to patients in the developed world. High mortality amongst AIDS patients in the developing countries could therefore be attributed to limited access to adequate health care (15). This is partly because of social stigma attached to people living with HIV/AIDS (16) and partly because of poverty, high cost of ARV treatment and lack of sufficient international financial aid to fund ARV treatment programme (17). Solving the problems of HIV/AIDS in Nigeria therefore, requires multisectoral collaboration especially from the private individuals and the organized private sectors to expand the scope of patients access to ARV drugs as it is currently being done in some African countries (18, 19).

In conclusion ARV drug was effective, albeit with minimal side effects, in stopping the clinical progression of HIV disease, it improved immunological and general well being of the patients thereby prolonging survival of people living with HIV/AIDS. Therefore we suggest; (i.) Early commencement of ARV drugs in all symptomatic HIV/AIDS patients, (ii) Treatment points of these should be increased from the current 25 trial centers to include all the general hospitals in the federation so as allow easy access to the drugs, (iii) Government should continue to make ARV drugs available at cheap and affordable cost, with inclusion of protease inhibitors in the treatment regimen, (iv) Government should sustain the current public awareness campaign about HIV/AIDS and continue the political and financial supports shown so far to ameliorate the suffering of Nigerians living with the virus, (v) Private individuals and organized private sectors should be encouraged to help fund ARV treatment programme.

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LIVER FUNCTION TESTS IN HIV-1 INFECTED ASYMPTOMATIC PATIENTS AND HIV-1 AIDS PATIENTS WITHOUT HEPATOMEGALY IN LAGOS, NIGERIA

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Hepatic functions were assessed by serum assays of albumin (ALB), total protein (TP), total bilirubin (TB), conjugated bilirubin (CB), serum activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma – glutamyl transferase (GGT) in 51 HIV-1AIDS patients, 38 HIV-1 infected asymptomatic patients and 56 age and sex matched healthy HIV negative controls. The mean ± SEM serum ALB concentration of 23.5 ± 1.2 g/L in AIDS patients was significantly lower ($p < 0.001$) than those of HIV-1 infected asymptomatic patients and healthy controls; 38.9 ± 3.1g/L and 39.4 ± 2.8g/L respectively. The mean ± SEM TB concentration of 17.8 ± 1.3 µmol/L in AIDS patients was significantly higher ($p < 0.01$) than 11.7 ± 1.1µmol/L observed in HIV-1 infected asymptomatic patients and 10.8 ± 2.1µmol/L in the controls. Similarly, there was a significant elevation ($p < 0.05$) in serum CB concentration of 6.5 ± 0.9µmol/L in AIDS patients compared to HIV-I infected patients of 3.8 ± 1.0 µmol/L and controls of 3.1 ± 0.8 µmol/L. The mean ± SEM ALT, AST, ALP and GGT activities (iu/L) of 48.7 ± 3.1, 54.3 ± 3.3, 84.8 ± 4.3 and 47.5 ± 4.1 respectively in AIDS patients were significantly higher ($p < 0.001$) than 21.3 ± 2.9, 25.6 ± 1.3, 56.4 ± 3.2 and 25.1 ± 1.7 respectively observed for the same enzymes in HIV-1 infected patients and 20.1 ± 3.1, 24.5 ± 2.6, 54.6 ± 4.3 and 24.2 ± 2.1 respectively in the controls. These results provide evidence to suggest that hepatic damage is greater in AIDS patients than in HIV-1 infected asymptomatic patients even in the absence of hepatomegaly. We conclude that this may be due to opportunistic infections that set in at the later part of HIV-1 infection (i.e. at AIDS stage) or increase severity of HIV-1 infection or both

Key Words: HIV-1 infected asymptomatic patient, AIDS, Hepatic functions

INTRODUCTION

Patients infected with Human Immunodeficiency Virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), display a broad spectrum of clinical manifestations ranging from asymptomatic state to life threatening symptomatic state characterized by opportunistic infections and malignancies (1).

The hallmark of HIV infection is the cytopathic effect on the CD4 bearing cells (Helper T4 cells) (2). Apart from these cells, other CD4 protein bearing cells such as macrophages, B-lymphocyte, microglial cells, haemopoietic stem cells, rectal mucosal cells, Kupffer cells and liver sinusoid epithelial cells are also affected (3) HIV is a pathogen that causes a variety of specific and non-specific defects in immune function that result in diverse clinical consequences. Studies in the USA and Europe (4) indicate that approximately 50% of HIV infected patients will develop AIDS by 10 years; 75% by 15 years and 90% by 20 years (4).

In about two weeks after infection with HIV, approximately 50% of patients will develop a viral illness that may resemble glandular fever, influenza or aseptic meningitis. In more severe cases,

hepatomegaly and raised transaminases may be detected (5, 6).

Hepatomegaly is a frequent finding in about two-third of AIDS patients and some abnormalities in liver function tests of HIV and AIDS patients have been reported (7, 8). The cause of this hepatomegaly has been ascribed to various mechanisms such as infection arising from HIV, hepatitis B, non-A non-B, sepsis, alcoholic liver disease and malnutrition (8-14). Although the presence of hepatomegaly indicates some degree of hepatocellular damage and or hepatobiliary obstruction in HIV-1 AIDS patients, it is not however clear whether hepatic pathology exists in HIV-1 infected asymptomatic and HIV-1 AIDS patients without hepatomegaly.

This study compares the biochemical indices of liver cell damage and hepatobiliary obstruction in HIV-1 infected asymptomatic and HIV-1 AIDS patients without hepatomegaly.

MATERIALS AND METHOD

The study was carried out after obtaining approval from the Ethical Committee of the Lagos University Teaching Hospital, Nigeria. Forty five AIDS patients and 38 HIV-1 infected patients were randomly selected from those seen in LUTH for this study. The HIV-1 infected asymptomatic patients consists 16 males and 22 females aged between 20-70 years from among those found seropositive by ELISA method (15) and confirmed by Western Blot (16). The full-blown HIV-I AIDS patients consist 19 males and 26 females aged between 20-70 years. Fifty six control subjects, 25 males and 31 females, were selected from healthy individuals matched for age and sex who were screened and found negative for HIV-I and II.

Subjects who had evidence of hepatitis B, non-A non-B, alcoholic liver disease, hepatomegaly, sepsis, malnutrition and those on cytotoxic drugs were excluded from the study. Screening for hepatitis was carried on each subject and control, using Wellcome ELISA kit. Those positive were excluded from the study.

About 5 milliliters of venous blood was collected from each of the subject and control and was allowed to clot and retract. Serum was obtained after centrifugation at 3,500 rpm for 10 minutes and the samples were stored frozen at – 20°C until analyzed. Lyophilized controls were obtained from Randox Laboratory. Analysis was carried out in batches with the aid of BECKMAN SYNCHRON (CX5 serial no 4562) autoanalyser, using appropriate control with each batch. Serum albumin was estimated based on the dye-binding method of Doumas, Watson and Biggs (17), total protein by the method of Doumas, Bayse and Carter (18), total and conjugated bilirubin by the method of Malloy and Evelyn (19), AST by the method of Karmen (20), ALT by the method of Wroblewski and LaDue (21), ALP by the method of Bassey *et al* (22) and GGT by the method recommended by IFCC Part 4 (23). Results obtained were subjected to statistical analysis using computer with EPI-INFO version 6.2 software. The Student's t-test was used to determine the differences between the means of the various groups.

RESULTS

Table 1 displayed the demographic characteristics of sample population while Table 2 displayed the summarized comparative mean ± SEM of hepatic biochemical parameters in the two groups of

subjects and the controls. The result shows a significant decrease ($p < 0.001$) in the mean serum concentration of albumin in AIDS patients when compared with healthy controls and HIV-1 infected asymptomatic patients. Also, a significant increase ($p < 0.01$) in serum level of total bilirubin was observed in AIDS patients compared to HIV-1 infected patients and healthy controls. Similarly, there was a significant elevation ($p < 0.05$) in the serum level of conjugated bilirubin in AIDS patients when compared to HIV-1 infected asymptomatic patients and the controls.

The mean serum activities of ALT and AST in AIDS patients were significantly increased ($P<0.001$) when compared with those of HIV-1 infected asymptomatic patients and the controls. The ALT and AST activities in the controls were not significantly different from those of the HIV-1 infected asymptomatic individuals. The mean serum activities of ALP and GGT in AIDS patients were significantly increased ($P<0.001$) when compared with those of HIV-1 infected asymptomatic patients and controls respectively. The mean serum activities of ALP and GGT in controls were not significantly different from those of HIV-1 infected asymptomatic individuals.

Table 1: Demographic characteristics of sample population

Age group (In years)	Control		HIV- 1 infected		AIDS	
	Male	Female	Male	Female	Male	Female
20 - 40	12	15	9	10	10	14
41 - 60	9	12	5	8	8	10
> 60	4	4	2	4	1	2

Table 2: Means ± SEM of hepatic biochemical parameters in HIV-1 infected Patients and AIDS patients without hepatomegaly, and controls

Parameter/Unit	Control	HIV-1 infected	AIDS		
Albumin (g/L)	39.4 ± 2.8	38.9±3.1	23.5±1.2	♦♦♦	***
Total Protein(g/L)	74.3 ± 5.1	73.6±6.3	72.9±4.8		
Bil. Total(μmol/L)	10.8 ± 2.1	11.7±1.1	17.8±1.3	♦♦	**
Bil. Conj(μmol/L)	3.1 ± 0.8	3.8±1.0	6.5±0.9	♦	*
ALT (IU/L)	20.6±3.1	21.3±2.9	48.7±3.1	♦♦♦	***
AST (IU/L)	24.5±2.6	25.6±1.3	54.3±3.3	♦♦♦	***
ALP (IU/L)	54.6±4.3	56.4±3.2	84.8±4.3	♦♦♦	***
GGT (IU/L)	24.2±2.1	25.1±1.7	47.5±4.1	♦♦♦	***
Alb./Globulin Ratio	39.4/34.9	38.9/34.7	23.5/49.4	♦♦	**

♦= P < 0.05; ♦♦= P < 0.01 and ♦♦♦ = P < 0.001------AIDS Vs Controls
*= P < 0.05, ** = P < 0.01 and *** = P < 0.001------AIDS Vs HIV-1 infected

DISCUSSION

In this study, the concentration of serum albumin was found to be low in AIDS patients compared to controls. This finding is similar to the study of Geffriand *et al* (24), who found that serum albumin was

significantly reduced in AIDS patients. But Cello (25) and Cappell (26) reported no significant reduction in albumin concentration when AIDS patients were compared with controls. In acute liver disease, there may be little or no reduction in plasma albumin because its biological half-life is about 20 days and also the fractional clearance rate is very low (27). Apart from chronic hepatic lesions, hypoalbuminaemia found in this group of patients, may be due to increased catabolism from tissue damage, inflammation, malnutrition, malabsorption syndromes and protein loss (28). It has been observed that majority of patients with AIDS who develop diarrhoea have some degree of malabsorption (29). All the AIDS patients in our study had diarrhoea and this may have synergistically contributed to hypoalbuminaemia resulting from reduced synthetic liver function.

The observed significant reduction in the mean ± SEM plasma albumin in AIDS patients compared to the control subjects suggests a chronic hepatic dysfunction. This is further corroborated by the significant elevation of the mean ± SEM plasma activities of ALT and AST in AIDS patients compared to the controls and HIV-1 infected asymptomatic group. This observation is in keeping with the works of other researchers (7, 25, 26, 30), who found similarly elevated ALT and AST in AIDS patients compared with controls.

The total protein found in both HIV-1 and AIDS patients does not differ from that of the control, but there is a reversal of albumin/globulin ratio in AIDS patients with a higher globulin fraction. A similar pattern has been reported by Mohammed (31) who noticed that AIDS patients have persistent generalized lymphadenopathy with hyperplasia of the B-lymphocytes in lymphoid follicles and polyclonal hypergammaglobulinopathy involving IgG, IgA and rarely IgM. Similar pattern of hypergammaglobulinaemia has been confirmed in AIDS patients in Northern Nigeria (32).

Our study showed a significantly elevated serum total bilirubin and conjugated bilirubin, which may indicate problem with excretory function of the liver. Though the subjects were not clinically jaundiced, this may be a pointer to hepatic pathology involving hepatocytes and its enzymes or an increase in the rate of haemolysis or a decreased rate of delivery of unconjugated bilirubin to the liver secondary to hypoalbuminaemia in this group of subjects.

Our observation tallies with the work of Dworkin *et al* (33) who found that significant rises in serum ALP and total bilirubin occurs during the course of AIDS in American patients. However Astagueau *et al* (34) in France, Cello *et al* (25) and Cappell *et al* (26) in America, reported that jaundice was rare in AIDS patients. Serum transaminases ALT and AST activities were significantly raised in AIDS patients compared to those of the healthy individuals, this is suggestive of an increased degree of hepatocellular damage in AIDS patients. This observation has been noted in previous studies carried out on American whites and blacks (12, 25, 26, 30), and by some other workers (24, 35, 36) who also found raised serum activities of ALT and AST in AIDS patients compared to healthy individuals. Opportunistic infections

of the liver such as *Mycobacterium avium-intracellulare*, *Mycobacterium tuberculosis*, Cytomegalovirus, *Cryptococcus neoformans*, and *Pneumocystis carinii* which are common complications in AIDS patients have been implicated as the cause of liver parenchymal cell damage (25).

Significantly elevated serum ALP and GGT observed in AIDS patients may be a pointer to a higher degree of damage that results in hepatobiliary obstruction in this group of subjects. Invasive and non-invasive diagnostic procedures have revealed and documented papillary stenosis, sclerosing cholangitis, cholecystitis as well as thickened gall bladder wall in a growing population of AIDS patients (14). All these structural abnormalities have been attributed to opportunistic infections. Our findings agree with the previous studies in America (13, 14, 25, 26, 30, 37), and some other parts of the world where a similarly elevated ALP and GGT in AIDS patients was found (35, 36, 38). Previous studies explained that this biochemical relationship is compatible with localized biliary obstructive lesions in liver due to localized CD4 site in the liver sinusoid epithelial cell surfaces (39, 40, 41).

Opportunistic infections in late stage of the disease (AIDS) may likely be the cause of hepatic damage in this study however the time of onset or degree of infection was not determined to establish whether difference in findings between asymptomatic HIV-1 infected and AIDS patients are due to severity of the condition. This possibility cannot be totally ruled out.

CONCLUSION

The significant reduction in the serum albumin is a strong pointer to the presence of chronic hepatic lesion in patients with AIDS although various other possibilities like diarrhoea were adduced in the discussion. Our result shows that hepatic lesion in AIDS patients without hepatomegaly affects both hepatocellular integrity and hepatobiliary obstruction with a greater severity than HIV-1 infected asymptomatic patients without hepatomegaly. We therefore conclude that the level of hepatic function distortion is greater in AIDS patients than in HIV-1 infected asymptomatic patients even in the absence of hepatomegaly. Further work need to be carried out to determine whether the level of distortion of hepatic function is similar in these two groups of patients in the presence of hepatomegaly.

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ORIGINAL ARTICLE

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HEALTH WORKERS' ATTITUDE AND PERCEPTION TOWARD ROUTINE PRE-MARITAL HIV SCREENING

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More than half of all new HIV infections occur among young adults, however, the rate of new infections among women surpasses men's especially in Sub-Saharan Africa. This rising infection rates, particularly among women, exposes children to increased HIV risk even before they are born. This descriptive cross-sectional study was conducted to determine the attitude and perception of health workers to routine pre-marital HIV screening that is currently practice by some religious institutions as part of initiative directed towards controlling the spread of the infection. Three hundred (300) self-administered questionnaires distributed to the health workers in their respective units were analyzed. Majority of the respondents 270 (90%) agreed that pre-marital HIV screening is necessary and advantageous to couples intending to get married. Although more than half of the respondents (56.7%) believed that the screening exercise is associated with some disadvantages, as many as 205 (68.3%) were in support of its enforcement for all couple. Majority 260 (86.7%) agreed that religious leaders/institutions have important role to play in HIV control and most of them 265 (88.3%) would prefer that Government health facilities be used as screening centres; and medical doctors should be the person to reveal the test results to the couples 275 (91.7%). About two-third of the respondents felt that the couples should initiates request for HIV screening, and on the issue of whether or not the marriage should be contracted following a positive result in one or both partners, 180(60%) respondents felt that the decision should be made by the couple. Counseling of couples before and after HIV screening, adequate training of health workers on HIV counseling skill and making HIV screening free to couple were suggested by the respondents as incentive that would enhance voluntary pre-marital HIV testing.

INTRODUCTION

More than 60 million people have been infected with HIV in the past 20 years and about half of them became infected between the ages of 15 and 24 (1). Today, nearly 12 million young people are living with HIV/AIDS, and young women are several times more likely than young men to be infected with HIV (2).

In about 20 African countries, 5% or more of women aged 15 to 24 are infected with HIV (3). Public health officials estimate that illnesses and deaths resulting from HIV/AIDS, to date, represent only 10% of the eventual impact (4, 5). It is also projected that by the year 2010, HIV/AIDS will reduce average life expectancy in some southern African countries to around 30 years (6). About 90% of the estimated 2.7 million children living with HIV/AIDS are living in Sub-Saharan Africa (2). The overwhelming majority of these children were infected from their mothers, during pregnancy, childbirth or breastfeeding (1).

Necessary control programme, therefore, needs to focus on preventing HIV among youths and adolescents and reduce transmission from mother to child. Early screening for HIV/AIDS is an important control strategy that is useful in case finding and public health surveillance. In case finding, the primary objective is to ascertain the HIV infection status of an individual for appropriate medical treatment or public health follow-up and action. The public health surveillance aimed at determining the prevalence, distribution and trends of HIV infection in a population (7).

In the last 2 years many couples have presented at the University of Ilorin

Teaching Hospital in Ilorin with request for HIV screening ordered by the marriage counseling unit of their religious institution as a precondition for their marriage. This action is, no doubt, an important initiative on the part of the religious institutions in the control of the deadly disease that target adolescents who are the most vulnerable group. Health workers have important role to play in this regard. Health workers are not only to support the initiative, but should champion the course through counseling of youths/adolescents to encourage them to undergo voluntary HIV test.

This study was carried out to determine the attitude and perception of trained health workers toward routine pre-marital HIV screening. As a major stakeholder/partner in any control programme, their views and opinion about pre-marital HIV screening will go along way to determine its success in term of public acceptability and the implementation.

METHODOLOGY

This descriptive cross-sectional survey was conducted at the University of Ilorin Teaching Hospital in Ilorin. All trained health workers were the target population of the study. Structured questionnaires were distributed to all relevant units/departments of the hospital. The self-administered questionnaire contained questions that elicited information on attitude and perception of the respondents towards pre-marital HIV screening. A total of 420 questionnaires were distributed to the staff in their various units/departments, 300 were properly completed and returned for necessary analysis giving a response rate of 71.4%.

Data analysis was done using the EPI-INFO computer software. Cross-tabulations were done where necessary and Chi-square analysis used to determine statistical significance of differences in some of the observations. Level of significance was set at P-value <0.05.

RESULTS

The age range of the respondents was 20-49 years with a mean of 32.8 ± 6.6. About a third of them (33.3%) were aged between 26-30 years, and their professional status is highlighted in Table 1. Females constituted 46.7% of the respondents while the rest 160(53.3%) were males. One hundred and fifty-five (51.7%) respondents were married, 100(33.3%) were single, 40(13.3%) separated, while only 5(1.7%) were divorced. Less than half of the total respondents (43.3%) were Muslims while 170(56.7%) were Christians.

Majority of the respondents 215(71.7%) were aware that some religious institutions are enforcing HIV screening on couples intending to get married. Most of them 270(90%) agreed that pre-marital screening is necessary and 280(93.3%) said it is advantageous to couples intending to get married. Although more than half of the respondents (56.7%) believed that the screening has some disadvantages, as many as 205(68.3%) were in support of the enforcement of the practice for people intending to get married (Table 2). While most of the respondents 260(86.7%) agreed that religious leaders/institutions have important roles to play in HIV control programme, majority 265(88.3%) preferred that Government health facilities be used as venue where HIV screening should be conducted; and 190(63.4%) felt that couples

should be the initiator of request for the screening. On the issue of decision on whether or not the marriage should be contracted following a positive result in one or both partners, 180(60%) were of the opinion that the couple should be allowed to decide. Medical doctors were mostly preferred by the respondents as people to reveal the test results to the couples 275(91.7%) (Table 3).

Among those respondents who believed that the screening is necessary and advantageous, a higher proportion of them were in support of the enforcement and were willing to encourage and counsel couples to undergo pre-marital HIV screening as against a lower proportion of respondents who believed the test is unnecessary nor advantageous and hence were not in support of it and would not be willing to encourage or counsel couples to go for the test. This difference is statistically significant (Table not shown). Sex, marital status and religion have no significant effect on whether or not the respondents had ever been screened before (Table not shown). Similarly, respondents' professional status did not make any significant difference on the support expressed for the enforcement of the pre-marital HIV screening or their willingness to counsel couples to undergo the test (Table 4).

The advantages of pre-marital HIV screening listed by the respondents included: opportunity to know HIV status of the couples, prevent HIV transmission to uninfected partner, early treatment/intervention for positive victims and to know couples that should not get married. The reasons against enforcing pre-marital HIV screening were ethical violation of couple's right, stigmatization associated with positive result, possibility of increase spread by partners testing positive and belief that marriage is a personal affair (Table 5). The reasons given by 50 respondents who were not willing to encourage or counsel couples on pre-marital HIV testing were lack of treatment for positive victims 29(58%), afraid of any involvement in disclosing positive result to victims 9(18%), fear of been involved in action that could lead to break in marital relationship 5(10%) and lack of personal interest on the issue 7(14%). The suggestions given by the respondents to ensure general acceptability and implementation included: pre-marital counseling of couples, making the test voluntary and free of charge for couples and training of health workers on how to carry out counsel to encourage and convince couples to voluntarily undergo pre-marital HIV screening (Table 5).

Table 1: Respondents' age distribution and profession

Age group (years)	**Frequency**	**%**
< 26	40	13.3
26 - 30	100	33.3
31 - 35	50	16.7
36 - 40	65	21.7
41 - 45	40	13.3
46 - 50	5	1.7
Total	**300**	**100**
Respondents' Profession	**Frequency**	**%**
Nurses/Midwives	120	40.0
Doctors	70	23.3
Lab Technologist/Technician	35	11.7
Pharmacists	35	11.7
Other Paramedics*	40	13.3
Total	**300**	**100**

*(Other paramedics = Health record Officers, Community health Officers, Physiotherapist and Medical Social Workers)

Table 2: Some of the respondents' views / opinion towards pre-marital HIV screening

	Frequency (%)		
Expressed view / opinion	**Yes**	**No**	**Total**
*Agreed that premarital HIV screening is necessary	270(90)	30(10)	300(100)
*Aware that premarital HIV test is been practiced	215(71.7)	85(28.3)	300(100)
*Premarital HIV screening is advantageous to couples	280(93.3)	20(6.7)	300(100)
*Premarital HIV screening has disadvantages	170(56.7)	130(43.3)	300(100)
*Support enforcement of premarital HIV screening	205(68.3)	95(31.7)	300(100)
*Willing to counsel couples on Premarital HIV screening	250(83.3)	50(16.7)	300(100)
* Religious institutions have role in HIV control	260(86.7)	40(13.3)	300(100)

Table 3: Respondents' opinion on where test should be carried out, who initiates and disclose test results and who makes the final decision on whether or not a couple should get married when one or both of them are positive for HIV

A. Health facilities where Pre-marital HIV test should be done:

Type of health facility	**Frequency**	**Percentage (%)**
Government health facilities	265	88.3
Missionary health facilities	20	6.7
Private health facilities	15	5.0
Total	**300**	**100**

B. Persons who should initiate pre-marital HIV test:

Category of persons	**Frequency**	**Percentage (%)**
Couples	190	63.4
Health workers	55	18.3
Religious Leaders	45	15.0
Parents	10	3.3
Total	**300**	**100**

C. Persons who make decision on whether a couple should get married or not after the test:

Category of persons	**Frequency**	**Percentage (%)**
Couples	180	60.0
Health workers	90	30.0
Parents	20	6.7
Religious Leaders	10	3.3
Total	**300**	**100**

D. Persons expected to reveal Pre-marital HIV test results to the couples:

Category of persons	**Frequency**	**Percentage (%)**
Medical Doctor	275	91.7
Laboratory Officers	15	5.0
Religious Leaders	10	3.3
Total	**300**	**100**

Table 4: Respondents' profession in relation to whether or not they support enforcing pre-marital testing and their willingness to counsel couples to undergo the screening

	Support enforcement of Pre-marital HIV screening		
Respondents' Profession	**Yes**	**No**	**Total**
Nurses/Midwives	75	45	120
Doctors	54	16	70
Lab Technologist/Technician	26	9	35
Pharmacists	25	10	35
Other Paramedics*	25	15	40
Total	**205**	**95**	**300**
		(P value =0.2182	X^2 = 5.75 df =4)

	Willingness to counsel couples to undergo Pre-marital HIV screening		
Respondents' Profession	**Yes**	**No**	**Total**
Nurses/Midwives	98	22	120
Doctors	59	11	70
Lab Technologist/Technician	30	5	35
Pharmacists	29	6	35
Other Paramedics*	34	6	40
Total	**250**	**50**	**300**
		(P value =0.9720	X^2 = 0.51 df =4)

*(Other paramedics = Health record Officers, Community health Officers, Physiotherapist and Medical Social Workers)

Table 5: Advantages of the screening, reasons against enforcement of the test and suggestions relating to pre-marital HIV screening given by the respondents. Advantages given by the respondents (Multiple responses; N=280)

Advantages	**Frequency**	**Percentage**
Knowing HIV status of the couples	190	67.8
Prevent transmission of HIV to uninfected partner	168	60
Early detection and treatment	78	27.9
Identification of couples who should not get married	31	11.1
Reasons against enforcement of pre-marital screening (multiple responses; N=95)		
Reasons given	**Frequency**	**Percentage**
Ethical violation of self right	42	15.0
May cause stigmatization/ discrimination	37	13.2
May increase spread by diagnosed victims	30	10.7
Marriage is purely personal issue	23	8.2
Suggestions to ensure acceptability and implementation (multiple responses; N=300)		
Suggestions	**Frequency**	**Percentage**
* Pre-marital counseling of couples	159	53
* Pre-marital HIV test should be voluntary	114	38
* Test should be free	108	36
*Post HIV test counseling for couples	63	21
* Training of health workers on HIV counseling	42	14

DISCUSSION

The HIV/AIDS epidemic in Nigeria has rapidly gained momentum and thus making the disease a major public health concern. The prevalence of HIV infection has increased from 1.8% in 1991 to 5.4 % in 1999. This prevalence although lower than those of neighbouring African countries, it

should be considered high in the context of Nigeria teeming population of about 109million (8). Several factors have been documented to contribute to rapid spread of HIV in Nigeria. These include increased sexuality among the youth, majority of whom had sex at early age (9, 10), widespread practice of polygamy or multiple partners and sexual networking; and high prevalence of untreated sexually transmitted infection (8, 11).

The National Action Committee on AIDS (NACA) was constituted in response to this HIV/AIDS epidemic, and the committee has several activities directed towards controlling the disease using a guiding principle of involvement of individuals, groups and communities in the prevention, care and support for HIV victims (12). It is therefore not a surprise that some religious institutions have champion actions directed towards HIV control through mandatory screening for couple who intend to get joined in marriage in such religious institutions or groups.

Routine HIV screening, no doubt, has many benefits, but its practice is still minimal in most countries due to several reasons such as lack of treatment for those who are positive, stigmatization and discrimination against victims and lack of confidentiality in handling of results (1). Hence not many people would want to undergo voluntary HIV screening including health workers themselves.

A study done among health workers to determine their willingness to undergo HIV testing showed that more than 25% of them were not willing to undergo the test even for no fee charge (13). In the present study about 90% of the health workers agreed that the procedure is necessary for couples and over 75% of these were willing to provide counseling and encouragement to couples so that they can have the test carried out. These positive attitudes among the health workers towards pre-marital HIV screening could be due to their educational and professional status / background.

Ninety-five respondents were not favourably disposed to enforcement of couples to undergo pre-marital HIV screening their main reasons were ethical violation of individual right and stigmatization. These are important points that must be borne in mind in HIV screening because one of the major principle of any health education programme is to encourage voluntary action or change rather than compulsion and to ensure behavioural change. In implementing pre-marital HIV testing couple should have adequate counseling on the need for and the benefit of the screening exercise and why they should voluntarily request for the test. Other reasons expressed by respondents who opposed routine pre-marital screening were factual. Lack of treatment modalities for positive victims is a major limiting factor to wide acceptability of any screening programme, since the essence of any screening is to make early diagnosis with a view to instituting immediate intervention or treatment so that the clinical course of the disease may be favourably altered thereby improving the outcome of the disease. Therefore availability of HIV care and treatment services would be a powerful incentive for people to seek counseling and HIV screening. Absence of such service or facility would surely discourage people from wanting to know their HIV status.

Counseling of couples before and after HIV screening, adequate training of health workers on HIV counseling and making the screening free were important suggestions raised by the respondents which, if properly incorporated into routine HIV screening would go a long way to enhance its acceptability and its implementation.

Policy makers should harness initiatives and activities of individuals health professionals, groups and organizations that are directed towards HIV control among youths and young adults so that our social norm of marital life would be encouraged and sustained, while addressing the scourge of HIV / AIDS among this vulnerable group.

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ORIGINAL ARTICLE

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SERO-PREVALENCE OF HEPATITIS C VIRUS AMOUNG PATIENTS ATTENDING STD CLINIC IN IBADAN, NIGERIA

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In the tropics, hepatitis C virus (HCV) seroprevalence ranges from < 0.2% in whole Africa. A strong association between HCV and hepatitis B surface antigen (HBsAg)-negative chronic liver disease and hepatocellular carcinoma has been described. Hepatocellular carcinoma (HCC) is one of the most common cancers among Africans, and in Nigeria by 1970 the estimated rate was 6.6 per 100,000 populations per annum. Sexual transmission was regarded as a minor cause of HCV, the degree of which has not been properly evaluated in most environments. Since it has been established that sexual transmission is an important mode of acquisition of the infection, we therefore set out to find the seroprevalence of HCV among 95 patients attending sexually transmitted diseases (STD) clinic in University College Hospital, Ibadan, Nigeria with a view to recommending preventive and control measures of HCV in our community. The sera collected from these respondents were used for screening for syphilis using the VDRL test, and for HCV antibodies using the MONOLISA anti-HCV (Sanofi, Pasteur France). Mid-stream urine was collected from all participants, and urethral swabs from all male participants while endocervical and high vaginal swabs were collected from female participants. Ulcer swabs were collected from those with genital ulcers. The prevalence of HCV infection was found to be 37.9% in patients presenting with STDs. This comprised 38.9% of males and 61.1% females. This prevalence rate is very high compared with the rate in the general population and other "high-risk" groups in previous studies in the same environment. Factors associated with HCV infection in this environment include high heterosexuality, high level of education, and previous instrumentations such as in scarifications and termination of pregnancy. Prevention and control of STDs will definitely reduce HCV infection and hence the attendant consequences, particularly hepatocellular carcinoma, in our environment.

INTRODUCTION

Hepatitis C virus (HCV) is a positive strand RNA virus (1). A strong association between HCV and hepatitis B virus has been demonstrated. They have the same route of transmission and these include sexual, parenteral and vertically from mother to child (2). In various studies all over the world, the HCV seroprevalence among heterosexual groups was 1.5% in Amsterdam, 0.4% in Germany, 0.5-1% among UK blood donors, 0.68% among French blood donors and 1.4% in United States (3). In the tropics, HCV seroprevalence ranges from < 0.2% in whole Africa (4).

Studies on the prevalence of antibodies against HCV revealed several high-risk groups, such as poly-transfused patients, haemophiliacs, patients treated by haemodialysis or surgery, and intravenous drug abusers (2). A strong relationship between HCV and hepatitis B surface antigen (HBsAg)-negative chronic liver disease and hepatocellular carcinoma has been described (3).

Although it has been established that sexual transmission is an important mode of acquisition of the infection (4), the degree of which has not been properly evaluated in most environments. It is therefore important to document the seroprevalence of antibodies against HCV in the at-risk group in our community. We therefore set out to find the seroprevalence of HCV among patients attending sexually transmitted diseases clinic in University College Hospital, Ibadan, Nigeria. In addition, the risk factors for the transmission of HCV will be evaluated.

PATIENTS, MATERIALS AND METHOD

Subjects

The participants were recruited from patients attending the clinic for Sexually Transmitted Diseases, Special Treatment Clinic (STC) of the College of Medicine, University College Hospital, Ibadan, Nigeria between September 1997 and August 1998. Criteria for enrolment in the study were age of 18 years and above who gave consent voluntarily, after a detailed explanation of the purpose and procedure of the study. Each participant completed a structured questionnaire to assess demographic characteristics, medical history and sexual and social behaviour. The participants were examined for STDs using a standard protocol.

Specimen collection

Five milliliters of venous blood was collected from each participant. The serum was separated from each blood sample and stored at -20°C until analyzed. The sera were tested for syphilis and anti-HCV antibodies. Mid-stream urine was collected from all participants; urethral swabs from all male participants while endocervical and high vaginal swabs were collected from female participants. Ulcer swabs were collected from those with genital ulcers.

Laboratory work

Direct microscopy was carried out for gonorrhea (men and women), trichomoniasis, candidiasis and bacterial vaginosis (women only). Routine cultures were done for gonococci, *Haemophilus ducreyi* and *Candida albicans*. Non-specific genital infections due to *Chlamydia trachomatis* and *Ureaplasma urealyticum* was by exclusion.

HCV ELISA serology

Antibody to HCV was detected using the MONOLISA anti-HCV (Sanofi, Pasteur France) [3]. Testing was carried out according to the manufacturer's instructions. This test is based on the use of a solid phase prepared with solid antigens; two recombinant proteins produced by *Escherichia coli* from clones selected in the nonstructural area of the hepatitis C virus genome (NS3 and NS4), two peptides coded by capsid area of the HC virus genome. Detection is with the goat ant-human IgG antibody purified by affinity chromatography and coupled to peroxidase.

Steps in the performance of the test include; i) the test sera and the control sera were added to the wells. If the antibodies to HCV were present, they would bind to the antigens fixed on the solid phase, ii) the peroxidase labeled antibodies to human IgG was added after a washing step. They in turn bound to the specific antibodies captured on the solid phase, iii) after removal of the unbound enzymatic conjugate, the antigen-antibody complex was revealed by addition of substrate, iv) after the reaction has been stopped, the absorbance values were read using a microplate reader at 492/620nm. The absorbance measured for a sample allowed the presence or absence of antibody to HCV to be determined. The colour intensity is proportional to the quality of antibody to HCV bound on the solid phase. The absorbance of the positive control was □ 0.900 □ 2.500, and the individual negative control was < 0.200. The presence or absence of antibodies to HCV is determined by comparing for each sample the recorded absorbance with that of the calculated cutoff

value. Samples with an optical density less than the cutoff value are considered to be negative with the MONOLISA anti-HCV (new antigens) test. Samples with an optical density higher than or equal to the cutoff value are considered to be initially positive. We considered a specimen positive only if all three tests results were greater than the cutoff.

Serological test for syphilis

Screening for syphilis was carried out using the VDRL (Welcome) assay and reactive or borderline results were confirmed by using the TPHA test.

Statistical analysis

The data were analyzed using EPI INFO Version 6.0 computer software and results expressed in tables.

RESULTS

The distribution of age, sex and marital status of the respondents is shown in Table 1. Of the 95 respondents, 46 (48.4%) were males and 49 (51.6%) females (M: F ratio of 1:1.1); 47 (49.5%) were single and 48 (51.5%) married; 73.7% were in age range 21 - 35 years. The sexual contacts and HCV status of the respondents is shown in Table 2. Of the respondents, 37.9% had sexual intercourse with spouse alone; 49.5% with other partners (these were single men); 8.4% with spouse and other partners (this include 13% of males and 4.1% of female); 4.3% of men had sexual intercourse with both spouse and Commercial Sex Workers (CSW). These differences are statistically significant. (P value = 0.0000036). Of these respondents, 36 (37.9%) were positive for HCV. This comprised of 14 (30.4%) males and 22 (44.9%) females.

Of the respondents, 14 (38.9%) of the 36 of those who had sexual intercourse with spouse alone were positive for HCV, these were all females; 22 (37.9%) of the 58 respondents with multiple partners were positive for HCV, (P value = 0.9506), 3 of 4 men (75%) who had relation with CSW had HCV. This difference is significant statistically (P value = 0.0271)

Of the respondents, 61.1% did not use any precaution; 31.6% used condom; 2.1% used pills. Six point three percent of the women were on IUCD (Table 3). These findings are statistically significant (P value = 0.0278). Of the 95 respondents, 38 (40%) had needle prick, 18 (47.4%) of which were HCV positive, 18 of 57 (31.6%) respondents without positive history of needle prick were HCV positive, a difference that is not statistically significant (P value = 0.2562).

Considering scarification, 32 of 69 (46.4%) respondents who were scarified were HCV positive; whereas only 5 of the 26 (19.2%) without scarification were HCV positive. This difference is statistically significant (P value = 0.0451). The history of abortion was given by 23 (47%) of the females, 6 (26.1%) of whom were HCV positive, a finding that is statistically significant (P value = 0.0164). The history of instrumentation was offered by 22 (45%) of these females, 6 (27.3%) of whom were HCV positive, whereas only one male had instrumentation. These differences are significant statistically (P value = 0.0322).

The analysis of level of education, sexual contacts and HCV status revealed that of the 47 who had sexual intercourse with other partners, 43 (91.5%) had secondary and tertiary education. Of the 36 who had with spouse alone, 4 (11.1%), 12 (33.3%), 6 (16.7%) and 14 (38.9%) had no formal, primary, secondary and tertiary

education respectively. The higher the educational level, the higher the HCV infection rate. These differences are statistically significant (P value <0.05) (Table 4). The diagnosis of the respondents is shown in Table 6.

Table 1: Age, sex and marital status

Age (Years)	Sex		Marital status		Total number
	Male	Female	Single	Married	
15 – 20	2	2	4	0	4
21 – 25	10	19	23	6	29
26 – 30	12	9	14	7	21
31 - 35	10	10	5	15	20
36 – 40	9	5	1	13	14
41 – 45	2	2	0	4	4
46 – 50	1	2	0	3	3
Total	**46**	**49**	**47**	**48**	**95**
%	48.4	51.6	49.5	51.5	100
P value	0.595	0.00000087			

Table 2: Sexual contacts and HCV status of respondents

HCV status	Sexual contacts						
	Husband alone	Wife alone	Partners	CSW	Spouse and Partner	Spouse and CSW	Total
Positive (%)	14 (56.0)	0	16 (34.0)	2 (100)	3 (37.5)	1 (50)	36 (37.9)
Negative (%)	11 (44.0)	11 (100)	31 (66.0)	0	5 (62.5)	1 (50)	59 (62.1)
Total	**25**	**11**	**47**	**2**	**8**	**2**	**95**
%	26.3	11.6	49.5	2.1	8.4	2.1	100

Table 3: Marital status, precaution and HCV

Marital status	Types of precaution used						HCV		
	Nil	Condom	Pills	IUCD	Others	Total	Positive	Negative	Total
Single	25	21	1	0	0	47	17	30	47
Married	33	9	1	3	2	48	19	29	48
Total	**58**	**30**	**2**	**3**	**2**	**95**	**36**	**59**	**95**
%	61.1	31.6	2.1	3.2	2.1	100	37.9	62.1	100
P value	0.02778						0.580		

Table 4: Education, sexual contacts and HCV

Level of education	Sexual contacts							HCV		
	Husband Alone	Wife alone	Other Partner	CSW	Spouse & other partner	Spouse & CSW	Total	Positive	Negative	Total
None	2	2	2	0	1	0	7	3	4	7
Primary	9	3	2	1	3	1	19	8	11	19
Secondary	5	1	20	0	4	0	30	11	19	30
Tertiary	9	5	23	1	0	1	39	14	25	39
Total	**25**	**11**	**47**	**2**	**8**	**2**	**95**	**36**	**59**	**95**
%	26.3	11.6	49.5	2.1	8.4	2.1	100	37.9	62.1	100
P value	0.03480284							0.04233256		

Table 5: HCV results of respondents

Specimen/Result	Specimen/Result	Specimen/Result	Specimen/Result
1 + -	25 +	49 +	73 -
2 -	26 -	50 +	74 +
3 -	27 -	51 +	75 + +
4 -	28 -	52 -	76 + +
5 + +	29 -	53 +	77 -
6 -	30 -	54 -	78 -
7 -	31 -	55 + +	79 +
8 -	32 -	56 -	80 +
9 -	33 -	57 -	81 -
10 -	34 -	58 -	82 +
11 +	35 + + +	59 +	83 +
12	36 -	60 + + +	84 +
13 -	37	61 -	85
14 -	38	62 +	86 +
15 -	39 -	63 +	87 -
16 -	40 -	64 -	88 +
17 -	41 +	65 +	89 -
18 -	42 +	66 + + + +	90 +
19 +	43 -	67 + +	91 -
20 -	44 -	68	92
21 -	45 -	69 +	93 -
22 + + +	46 -	70 +	94 -
23 + + +	47 +	71 -	95
24 -	48 -	72 -	
Controls	**i) Positive + + +**	**ii) Negative**	

Of the 95 specimens 36 (37.9%) had antibodies against HCV.

Table 6: Diagnosis of the respondents

Diagnosis	H C V		Total
	Positive	Negative	
Non-Specific Urethritis	6	12	18
Non-Specific Cervicitis	5	5	10
Gonococcal Urethritis	3	6	9
Gonococcal Cervicitis	0	1	1
Bacterial Vaginosis	5	8	13
Vaginal Trichomoniasis	1	3	4
Vaginal Candidiasis	2	4	6
Tinea cruris	1	0	1
Genital warts	1	3	4
Genital herpes	2	0	2
UTI	0	1	1
Chancroid	0	3	3
Schistosomiasis	0	1	1
Vaginal Candidiasis & Gonococcal Cervicitis	0	2	2
Non-Specific Urethritis & Tinea cruris	0	2	2
Bacterial Vaginosis & Genital warts	0	1	1
Bacterial Vaginosis & Vaginal Candidiasis	4	2	6
Bacterial Vaginosis & Bartholin cyst	1	0	1
Bacterial Vaginosis & Gonococcal cervicitis	1	1	2
Bacterial Vaginosis & Genital ulcer	1	0	1
Bacterial Vaginosis & Genital herpes	0	1	1
Bacterial Vaginosis & Genital warts & Trichomoniasis	0	1	1
Gonococcal urethritis & Conjunctivitis	1	0	1
Non-Specific Urethritis & Genital Ulcer	0	1	1
Venerophobia	1	1	2
HIV/AIDS	1	0	1
Total	**36**	**57**	**95**
%	37.9	62.1	100

DISCUSSION

People in the age range 21 - 35 years form the greatest percent of attendees in STD clinic (73.7%) a finding that is similar to those of previous workers (6, 7). This is the age range when sexual activity is highest. In this study, the seroprevalence of HCV infection was found to be 37.9% in

patients attending STD clinic in Ibadan, Nigeria. This comprised 38.9% of males and 61.1% females. This prevalence rate is very high compared with the rate in selected risk groups in previous study in the same environment in which an estimated prevalence of 11% of anti-HCV antibodies was found in doctors and dentist; 10.9% of non-hepatic patients and 18.7% of patients with hepatocellular carcinoma (8). Data on the HCV infection in Africa are still incomplete and somewhat contradictory, some sero-epidemiological studies have claimed a prevalence of anti-HCV antibodies in healthy subjects ranging from 4% to 12.5%; others have found it to be as low as that reported from western Europe or North America, where serological evidence of contact with HCV is found in < 1.5% of blood donors or general population (9).

Our study population is that with high sexual exposure, a fact that supports sexual route as an important mode of transmission (2). Of these respondents, 37.9% had sex with spouse alone, while 62.1% had with multiple partners. Those with multiple partners had high prevalence rate than those with single partners. Hence heterosexuality is an important predator of HCV infection, a finding that occurs is HIV infection. Therefore avoidance of heterosexual behaviour will help to reduce HCV infection. Of the respondents, only 31.6% used barrier method during sexual intercourse, and these are likely to be protected from STI including HIV and HCV infections. The remaining 68.4% are likely to be exposed. Scarification, a common practice in our environment was found to be an important factor in the transmission of HCV. During scarification, non-sterile equipments are often used thereby aiding transmission. Hence scarification is a very strong factor in this environment.

The history of abortion was given by 23 (47%) of the females, 6 (26.1%) of whom were HCV positive, a finding that is statistically significant (P value = 0.0164). Just like scarification, instrumentation as in termination of pregnancy (TOP) is an important factor associated with HCV infection. The instruments used are either not sterilized or inappropriately sterilized by the quacks, who are the main actors of TOP in our environment. Studies on the prevalence of antibodies against HCV revealed several other "high-risk" groups, such as polytransfused patients, haemophiliacs, patients treated by haemodialysis or surgery and intravenous drug abusers (10).

The higher the educational level, the more likely the sexual adventure. This adventure is likely to involve multiple partners, and may explain the higher rate of HCV infection with increasing level of education. The higher level of education is prone to sophistication involving sexual exposure and hence HCV infection)

Since a strong association has been established between HCV chronic liver disease and hepatocellular carcinoma, preventive and control measures for STDs/HIV/AIDS will reduce the incidence of these liver diseases in our environment. These steps include 1) Primary prevention activities, the only strategies that can have an effect on those presently incurable STDs resulting from viral infections and these involve safer sexual behaviour (abstinence, being faithful to one faithful sexual partner, use of condom for penetrative sexual acts).

2) Secondary prevention activities, which involves adequate management of cases since "one person treatment and cure for STD is primary prevention for a potential contact" (11).

CONCLUSION

The prevalence of HCV infection was found to be 37.9% in patients presenting with STDs. This comprised 38.9% of males and 61.1% females. This prevalence rate is very high compared with the rate in the general population and other "high-risk" groups in previous studies in the same environment. Factors associated with HCV infection in this environment include high heterosexuality, high level of education, and previous instrumentations such as in scarifications and termination of pregnancy. Prevention and control of STDs will definitely reduce HCV infection, and hence the attendant consequences, particularly hepatocellular carcinoma in our environment

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PREVALENCE OF MEASLES NEUTRALIZING ANTIBODY IN CHILDREN UNDER 15YEARS IN SOUTHWESTERN NIGERIA

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The immune status of children under 15 years in the Southwestern region of Nigeria against measles virus was determined using the neutralization test with a view to assessing the herd immunity to the virus in these communities. A total of 256 serum samples collected from children were tested by the beta method of neutralization. Forty (15.6%) of these samples were found to be positive at a titre of 1:256, 35 (13.7%) at 1:128, 36(14.1%) at 1:64, 37(14.5%) at 1:32, 38 (14.8%) at 1:16, 27 (10.5%) at 1:8 and 16 (6.3%) at 1:4. Twenty-seven (10.5%) of the 256 samples had no detectable antibody to the measles virus. There was no significant relationship between the antibody titre to measles virus and the gender of the children ($p > 0.05$). Also, there was no significant difference using Chi square analysis between the neutralizing antibody titres and the age of the children ($p > 0.05$). All the children whose samples were tested were vaccinated against measles as attested to by their parents. However, the vaccination does not seem to protect all the children, for some of them had no detectable neutralizing antibody while some had low neutralising antibody titre. In Nigeria, where only a single dose of measles vaccine is given at 9 month, measles may remain a serious threat to the children population with its attendant high morbidity and mortality.

Keywords: Prevalence, Neutralizing antibodies, Children < 15years

INTRODUCTION

Measles is one of the common childhood exanthemata, characterized initially by respiratory symptoms, cough, coryza, conjunctivitis, Koplik spots, and maculopapular rash (1). It is the greatest killer of children in history and has been responsible for severe epidemics throughout the world. It still affects about 50 million individuals and causes up to one million deaths in developing countries annually (2). The disease ranked as one of the major causes of childhood mortality with about 36 million cases occurring yearly and a mortality of about one million cases occurring each year worldwide (3). The morbidity and mortality figures were three times higher before the introduction of an effective vaccine in 1962 (4).

The introduction of the live attenuated measles vaccine in industrialized countries since the 1960's had largely contributed to the control of the disease in these countries (5). However, due to low vaccine coverage, sporadic cases and outbreaks continue to occur among school age children (6). Also, the interference of transplacentally acquired maternal antibody with measles vaccination contributes to the vaccination failure (7).

The objective of this study is to determine the level of neutralizing antibody to measles in children below 15 years of age, an indication of their level of protection against measles virus infection.

MATERIALS AND METHODS

The subjects used for this study were children below 15 years of age in Atiba,

Ibadan North, Osogbo and Olorunda local government areas of Oyo and Osun states of Nigeria, which are representative of the Southwestern region of Nigeria. They were recruited from among the children who visited the state hospitals for treatment, having sought the consent of their parents, who were also asked to fill a questionnaire on behalf of these children. The questionnaire contained information about their demographic data and vaccination records.

About 5 milliliters of venous blood was collected from the antecubital vein of each child. The blood was allowed to retract and then centrifuged at 1500 rpm, and the sera collected were stored at -20°C until tested. Each of the serum samples was tested for neutralizing antibodies to measles using the beta neutralization method of varying serum constant virus (8).

RESULTS

A total of 256 serum samples from children in Osun and Oyo States were tested. Of these, 137 (53.5%) were from children in Osun and 119 (46.5%) were from children in Oyo state. One hundred and fifty one (59%) of the samples were from males while 105 (41%) were from females. Overall, 27 (10.5%) of the samples had no detectable neutralizing antibody to measles virus while 81 (31.6%) had detectable neutralizing antibody less than a titre of 1:32. One hundred and forty eight (57.9%) samples had a titre above 1:32.

Table 1: Distribution of neutralizing antibody titres of children used for the study

Titre	Number	Percentage
<4	27	10.5
4	16	6.3
8	27	10.5
16	38	14.8
32	37	14.5
64	36	14.1
128	35	13.7
256	40	15.6
Total	**256**	**100**

Table 2: Age distribution of children used for the study

Age(years)	Number tested	Number positive (%)	Number negative (%)
1-5	111	97 (87.4)	14 (12.6)
6-10	119	110 (92.4)	9 (7.6)
11-15	26	22 (84.6)	4 (15.4)
Total	**256**	**229 (89.5)**	**27 (10.5)**

$X^2 = 2.36$ df = 2 $p > 0.05$

Table 3: Comparison of the sex distribution with the neutralizing antibody titre

Titre	Male	Female	Total
>4	16	11	27
4	9	7	16
8	18	9	27
16	19	19	38
32	26	11	37
64	20	16	36
128	23	12	35
256	20	20	40
Total	**151**	**105**	**256**

$X^2 = 0.0000$ df = 1 $p > 0.995$

Table 4: Comparison of the neutralizing antibody titre and the age distribution

Titre/ Age yrs)	<4	4	8	16	32	64	128	256	Total
1-5	14	11	9	12	15	18	16	16	111
S 6-10	9	5	14	22	18	14	16	21	119
11-15	4	0	4	4	4	4	3	3	26
Total	**27**	**16**	**27**	**38**	**37**	**36**	**35**	**40**	**256**

DISCUSSION

Antibodies to the haemagglutinin (H) protein are the primary antibodies measured by neutralization of virus infectivity in tissue culture (9) just as Haemaglutination Inhibition (HI) test is used to measure the antibodies to this H-protein. Neutralizing antibodies play a very important role in preventing re-infection; therefore, the neutralization test is most often used to evaluate vaccine responses and access susceptibility to measles.

The result of this study shows that there is a high prevalence of measles neutralizing antibody in children in the Southwestern Nigeria. Two hundred and twenty nine (89.5%) had detectable neutralizing antibody to measles and 27 (10.5%) had no detectable neutralizing antibody i.e. are seronegative to the neutralization test. The high titres found in the age group 1-5 years can be due to either vaccination or immunity from a previous infection since many of the children had history of measles infection in this age group. This correlated with the fact that the disease in West Africa mostly occurs in infants less than 2 years old (10).

The gender distribution did not show any significant difference between males and females ($p > 0.05$). Although, the level of the neutralizing antibody dropped as age advances (11-15 years) in this study, the level was not significantly different from the lower age groups. The drop as the age advances has been said to be an indication that immunity conferred by vaccination wanes with time, as such older children could be exposed to infection if they are not given a booster dose of the vaccine. This is because the level of immunity elicited by live measles vaccine tends to diminish over the years especially within populations with little exposure to measles virus and minimal opportunity for the antibody titre boosting in association with sub clinical re-infection (11).

In a study by Aiki-Raji (12), measles virus was isolated from an 8 year old child. This was attributed to either a waning in the immunity of the child or deficiency in the seroconversion to the vaccine earlier given. A good percentage of the parents claimed that their children have been vaccinated, however, with the report of 10.5% of these children not having detectable neutralizing antibodies, it could be deduced that there is no proper seroconversion in those children with low titres. This could have been influenced by factors such as poor nutritional status of the children, diminished potency of the vaccine used as a result of poor handling of the vaccine before its eventual administration to the children.

CONCLUSION

The result obtained in this epidemiological study of measles in Southwestern Nigeria taking Oyo and Osun states as a case study, shows that the incidence of measles among the population may be directly related to the level of immunity to the virus. It is obvious that vaccination does not protect many of the children as many with documented records of measles vaccination still had low neutralizing antibody titres. The reasons for this may include low vaccine potency, poor vaccine handling and logistics of vaccination (13). With the vaccination regime being used in Nigeria, which is given in a single dose without any booster dose, measles may remain a serious threat to the children population with its attendant high morbidity and mortality. It is of note that exclusive breastfeeding does not protect the child adequately because breast milk contains minimal antibody to measles (14). The effect of this is quite pronounced as an opportunistic period of virus infection is created since children are often vaccinated against measles at 9 months of age in developing countries. Moreover, findings demonstrate that the decay of the passive maternally acquired measles antibody occur more rapidly than expected resulting in the susceptibility to measles virus in most infants (15). Vaccination has been observed to be the most effective means of controlling measles globally (16), therefore, effective vaccination campaign should be done to combat this disease among children.

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ORIGINAL ARTICLE

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SEXUALLY TRANSMITTED INFECTIONS IN OBAFEMI AWOLOWO UNIVERSITY TEACHING HOSPITAL, ILE-IFE, NIGERIA: A DECADE OF CLINIC EXPERIENCE

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Sexually transmitted infections (STIs) remain cosmopolitan in all societies of the world and in some cases assume epidemic proportions. These infections are common infectious diseases nowadays, with an annual incidence of more than 200 million cases a year. Venereal pathogens continue to increase in number and the spectrum of pathogens has limitless elasticity. While genital discharge and ulceration are common presenting symptoms, unusual findings on examination and investigation are not uncommon. We assessed our clinic experiences during the first ten years in an STI clinic. Salient findings are that 85% of all patients seen have an STI. The breakdown of infections revealed that *Candida albicans* was the most common venereal pathogen accounting for 24% while *Neisseria gonorrhoeae* accounted for about 18.0%. *Sarcoptes scabiei* and *Phthirus pubis* causing scabies and pediculosis accounted for 1.8% and 0.3% respectively. As commonly established, the age bracket 19 to 39 years was clearly the age group in which sexually transmitted infections were mostly diagnosed. In a control programme, this age group should be targeted, while there is the need to continue to stimulate awareness of both the general public and health workers at all levels on the problems of sexually transmitted infections, the scourge of all ages.

Keywords: Sexually transmitted infections, venereal pathogens, clinic experience, control awareness

INTRODUCTION

Sexually transmitted infections (STIs) remain cosmopolitan in all societies and in some cases assuming epidemic proportions (1-3). The WHO reported that STIs are the most commonly reported infectious diseases today It has been estimated that more than 200 million cases occur each year (4, 5). The range of pathogens that are known to be spread by sex continues to increase. In recent years, there has been an increase in viral conditions particularly herpes simplex virus (HSV) and human papilloma virus but a seeming decrease in syphilis and gonorrhoea (6). The recognition of human immunodefieciency virus (HIV) and the acquired immune deficiency syndrome (AIDS) has increased awareness of STDs.

In a typical STI clinic, two of the most common presenting symptoms are genital discharge (urethral and vaginal) and genital ulceration (7). Nevertheless, unusual findings on examination and investigations are not uncommon (8).

The dynamic factors in the spread of STIs are the acquisition of infection from one partner and its transmission to another. This depends on availability of partners which increases with population movement including migration from rural to urban areas, worldwide travel as well as relocation of whole populations due to wars and natural disasters. Social factors which promote the spread include affluence, alcohol, leisure, personal freedom, prostitution (commercial and clandestine) and ignorance (9). All socio-economic groups acquire STIs; even the unborn child is not spared (10).

Our experience in an STI clinic during a decade (June 1991-2001) are presented and discussed in this paper.

MATERIALS AND METHOD

The records of patients attending the STI clinic since its inception in June 1991 up to the end of June 2001 were retrieved and analyzed. The clinic is a referral clinic and patients are received from the various medical and surgical specialties especially the general outpatient department (GOPD). We collated demographic information on the patients which include age, sex, occupation, tribe and marital status and analyzed the results of laboratory investigations that were conducted. The aim was to determine the prevalence of venereal pathogens encountered as well as highlight the pattern of STIs.

Routinely in the clinic, general physical examination was done on each patient with special attention to the genital area. The routine laboratory investigations performed included microscopy, culture and sensitivity of urethral swabs from males, cervical and high vaginal swabs from females (occasionally urethral swabs were collected from female patients when indicated). Eye swabs were collected from patients with discharge from the eyes. Urine samples for microscopy were collected from both genders while the 3 urine test was performed only in male patients. All the patients attending the clinic for the first time had their blood collected and tested for antibodies to *Treponema pallidum*. All microbiological investigations were done according to standard procedures (11). Specialized investigations such as intravenous pyelography were performed when indicated.

Preliminary reports of smears of urethral, cervical and high vaginal specimen examinations (wet preparation and Gram stain) were obtained on the first day of attending the clinic. The results were used to start treatment when indicated. Patients with acute gonococcal urethritis, gonococcal cervicitis and gonococcal ophthalmia neonatorum were reviewed in 72 hours (third day) with the culture results. Patients were thereafter followed up for 1, 2 and 3 weeks after the second visit to the clinic. The test of cure was performed in treated patients 2 weeks after completion of treatment.

RESULTS

During the period June 1991 to June 2001, a total of 1264 new patients were seen at the STI clinic. These were made up of 491 (38.8%) males and 773 (61.2%) females. The youngest patient was 2 years old while the oldest was 62 years (Table 1).

Table 1: Age and sex distribution

Age in year	No of attendees	Male	Female
Less than 10	5	1	4
10-14	2	2	-
15-19	84	23	61
20-24	383	80	302
25-29	228	122	106
30-34	249	99	150
35-39	186	92	94
40-44	55	30	25
45-49	24	13	11
50-54	15	7	8
55-59	18	10	8
60	16	12	4
Total	**1264**	**491(38.8%)**	**773(61.2%)**

The occupational distribution of the patients is as shown in Table 2.

Table 2: Occupational distribution

Occupational grouping	Frequency	Percentage
Skilled	186	14.72
Unskilled	266	21.04
Professional	793	62.74
Unspecified	19.	1.50
Total	**1264**	**100**

Majority of the clinic attendees were professionals (62.7%), followed by unskilled workers (21.04%) and skilled workers (14.72%) while 1.50% of the patients did not have any specific occupation. Tribal distribution revealed a mixed population with a Yoruba majority 73.6%, followed by Hausa/Fulani 11.8%, and Igbo and other Nigerian tribes and a few non-Nigerians, made up the remaining 14.6%.

A total of 978 diagnoses were recorded, 3 of these were by direct observation of the ectoparasite (*Phthirus pubis*) (Table 3). Two hundred and eighty six (23%) patients did not have specific STI, among who were 252 without a proven STI and 31 cases of venerophobia. The breakdown of the diagnoses revealed that candidal infections were in the majority (24.3%) while gonorrhoea ranked second with 17.59%. Non-specific urethritis (NSU) and non-specific genital infections (NSGI) accounted for 14.93%, pediculosis had the lowest prevalence of 0.3% (Table 3).

Table 3: Disease Frequency

Diagnosis	No of patients		Total	%
	Male	Female		
Candidiasis	6	232	238	24.33
Gonorrhoea	143	29	172	17.59
NSU/NSGI	130	16	146	14.93
Trichomoniasis	1	67	68	6.95
Genital warts	33	24	57	5.83
Wart and HIV	-	4	4	0.41
Bacteria vaginosis	-	51	51	5.21
Herpes genitalis	32	16	48	4.91
Tinea cruris	20	5	25	2.56
LGV	17	6	23	2.35
Chancroid	17	5	22	2.25
Scabies	18		18	1.84
HIV	5	14	19	1.94
Pediculosis	1	2	3	0.31
Venereophobia	27	4	31	3.17
Others	12	41	53	5.42
Total	**462**	**516**	**978**	**100**

DISCUSSION AND CONCLUSION

Vaginal candidal infection is the most prevalent STI in this study (24.3%) followed by gonorrhoea, NSU/NSGI and trichomoniasis with prevalence rates of 17.6%, 15% and 6.9% respectively. Pediculosis had the lowest prevalence of 0.3%. This pattern of distribution of cases of STIs is similar to that reported by other workers (12, 13) here in Nigeria. Odugbemi *et al* (13) in their series in Ilorin, North Central Nigeria, reported NSGI as the commonest condition encountered in the STD clinic, accounting for 21.8% of cases, followed by candidiasis 19%, gonorrhoea 14.3% and trichomoniasis 8.7%. Outside Nigeria, other workers (14-16) have reported similar distribution of STIs. Fonck *et al* in their study to determine the prevalence of STDs and cervical dysplasia in Nairobi, Kenya found that candidiasis was the predominant infection, accounting for 35% of all STIs.

Significantly, no case of syphilis was seen at the clinic. This is not surprising as others have also reported low incidence for this spirochaetal infection. Nagot *et al* (17) in Burkina Faso observed in their study that there is an important decline in classic bacterial STIs such as syphilis. Outside Africa, Claeys *et al* (18) reported 0.7% of 1185 women being seropositive for syphilis. The low prevalence observed could be due to the fact that the majority of STI cases receive treatment either at the primary health care level or in the private clinics or procure off-counter prescription before attending the STI specialist clinic. *Treponema pallidum*, the causative agent of syphilis is highly susceptible to a variety of drugs such as penicillins and macrolides,

which are readily available without a physician's prescription.

Scabies and pediculosis have prevalence rates of 1.9% and 0.3% respectively. Though these rates appear low, association between these organisms and STIs is emphasized. Infact, the United Kingdom national guidelines (19) on STIs have recommended screening for other STIs in patients with scabies attending the 'genitourinary medicine' clinic.

It is interesting to note that 31 (3.2%) of all the patients seen at our clinic had venereophobia. The majority of these patients, because of their unusual fear of STD, use various self-prescribed antimicrobial drugs including gentamicin, tetracycline, chloramphenicol, ciprofloxacin, penicillins and some cephalosporins. The widespread practice of indiscriminate use of antimicrobial agents only enhances the chances of survival of resistant pathogens in the communities. It was also observed that laboratory reports, which should only be used as a guide for prescribing drugs by physicians, now serve as a shopping list for drugs by patients especially those with venereophobia.

High incidence and prevalence of STIs has usually been reported in prostitutes or commercial sex workers (CSW). Though more females were seen during the period reviewed, none admitted to being a CSW. There is a strong stigma on prostitution in the local African community. It was also noted that none of the men admitted to being a homosexual. The level of sexual permissiveness in the society is not high enough to encourage a male Nigerian to admit being a homosexual. The reported cases of HIV with genital warts were low 0.4%, which might be due to the fact that management of HIV/AIDS patients is multidisciplinary.

Attendance at the clinic appears generally low and this could be attributed to two major factors, which include stigma and fear of loss of dignity. However, the study has again affirmed that STIs are prevalent in the sexually active population in any given community. Eighty two point seven percent of all cases of STI in this study were diagnosed in the age bracket 19-39 years, a pattern that is consistent with report of other workers (13, 16).

In conclusion, the problem of sexually transmitted infection is ever present and there is need to continue to stimulate the awareness of both the general public and health workers at all levels of health care delivery as a sure means of achieving control.

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REVIEW ARTICLE

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THE ROLE OF CLINICAL PATHOLOGISTS IN THE MANAGEMENT OF MALE INFERTILITY

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Male infertility is receiving increasing attention in Africa as up to 50% of cases of infertility are ascribed to it. In the management of this condition, the clinical laboratory plays a crucial role especially in the proper identification of causes of infertility. The role of the pathologists in this respect stems from the choice of laboratory equipment, reagents, type of samples needed, proper sample collection and its preparation. Added to these functions, the pathologist should help in the proper selection of required tests so as to ensure optimum diagnosis and treatment efficiency. Semen analysis is the first test required in the laboratory assessment of the infertile male. The obtained spermogram serves as the pivot for further tests which include hormonal assays, tissue studies and karyotyping. There is a disturbing high prevalence of azoospermia among Nigerians. The expected laboratory evaluation and treatment of such patients and others with abnormal spermogram are discussed in this review.

Key words: Male infertility, review.

INTRODUCTION

Infertility is a worldwide problem (1) that is attracting the attention of various researchers (2), even in Africa (3). Before now, men who were sexually potent were also thought to be fertile. This erroneous position has been discarded as male factors have been shown to be responsible for between 30-50% of cases of infertility (4, 5). Little wonder that 1% to 30% of children cannot be genetically matched to their presumptive fathers (6).

It has been suggested that the fertility prowess of fertile men decreases with time, since semen quality has been shown to deteriorate by as much as 3% per year (2). This probably contributes to the observation that the prevalence of male infertility in Nigeria is on the increase (7).

Generally, laboratories contribute to the actual diagnosis of infertility in over 50% of couple's investigated (8). It is very important that the right diagnosis is made early, as this is a crucial factor in the management of male infertility (9). In our environment, the use of empirical treatment by doctors, in the management of infertility has yielded dismal results (10). The import of this is that the clinical pathologists' role in the management of male infertility is enormous.

PRE-ANALYTICAL CONSIDERATION

In order to ensure quality laboratory services in male infertility management, the pathologist should be involved in patient preparation before laboratory analyses. This is in addition to his role in ensuring provision of adequate laboratory reagents and equipment, technical expertise and comfortable environment for the patients.

The pathologist should ensure that reference ranges, conditions of sample collection, sample transportation and specimen preservation, are in keeping with proper clinical applications. For example, the serum hormonal kits should be selected considering the coefficient of variation which should be 10% or less (11).

The kind of sample and frequency of sampling should be stated. Unlike in females, there is no cyclical secretion of luteinising hormone (LH), follicle stimulating hormone (FSH); prolactin (PRL) and testosterone in males, hence a single blood sample suffices for hormonal investigation of infertile males (12). In semen analysis of such men, more than one sample, collected at least one week apart after 3-5 days of sexual abstinence, is required to establish abnormal spermogram.

Sample preparation is important in the laboratory evaluation of these patients. While semen sample should be analyzed within an hour of collection, testicular biopsy specimen can be preserved. Such biopsy specimens are better kept in Zenker's solution or Bouin's fixative (13). In taking such materials, an open biopsy is more satisfactory than a punch biopsy (14). For hormonal assays, it is important that the samples are not kept for too long, and they should be devoid of gross haemolysis and lipaemia. In places where saliva is used for steroid estimation, the relevant analytical and interpretive expertise must be available (15).

ANALYTICAL/POSTANALYTICAL ROLES

When a representative sample is properly taken and well processed, the clinical pathologist will then interpret the findings of laboratory analyses. Furthermore, he/she advices the clinicians on further tests that might be of benefit to the management of such patients. The first test that should be done for suspected infertile male is semen analysis. The observed spermogram not only give a useful clue to the fertility status, it also could be a pointer to an obstructive disorder in the male.

Semen analysis parameters are commonly based on the WHO guidelines (16). The parameters assessed are appearance, consistency (viscosity), volume, and pH. These are followed by microscopic examination for sperm motility, morphology, concentration, and presence of cells other than spermatozoa (16, 17). In some places, computer assisted semen analysis (CASA) is readily available. Some of the CASA systems are coupled with video technology and sophisticated microcomputers to allow for automatic image digitalization and processing. This technology is thought to be more objective in measurement of seminal parameters than the subjective measurement of the standard traditional semen analysis. It also permits the added measurement of linearity, curvilinear velocity, straight line velocity and flagellar beat frequency of spermatozoa (18). Other seminal fluid analyses include sperm sperm-cervical mucus interaction, sperm penetration assay, acrosome evaluation and hypo-osmotic swelling test (18).

WHO defines normal ejaculate as sperm concentration of $\geq 20 \times 10^6$ spermatozoa/ml, with ≥ 50% with forward progressive motility or ≥ 25% with rapid progressive motility in 60 minutes of ejaculate and with ≥ 30% with normal morphology (16) (Table 1). But ideally, each

laboratory should set its own normal values, reflecting the specific population analyzed. For our own environment, the values commonly applied are shown in the work of Nkposong (19, 20) (Table 2). When the result of such analyses deviates considerably from those of a large population of tested men, then sub-fertility is likely (17).

Table 1: Normal values of semen variables (WHO, 1992) (16)

Standard tests	
Volume:	2.0 ml or more
pH:	7.2 – 8.0
Sperm concentration:	20 X 10^6 spermatozoa/ml or more
Total sperm count:	40 X 10^6 spermatozoa/ejaculate or more
Motility:	50% or more with forward progression or 25% or more with rapid progression, within 60 minutes of ejaculation
Morphology:	30% or more of normal forms
Vitality:	75% or more live i.e. excluding dye
White blood cells:	Fewer than 1 X 10^6/ml
Immunobead test:	Fewer than 20% spermatozoa with adherent particles
MAR test:	Fewer than 10% spermatozoa with adherent particles
Optional tests	
Alpha-Glucosidase (Neutral):	20 mU or more per ejaculate
Zinc (Total):	2.4 µmol or more per ejaculate
Citric acid (Total)	52 µmol or more per ejaculate
Acid phosphatase (Total):	200 U or more per ejaculate
Fructose (Total):	13 µmol or more per ejaculate
Nomenclature of semen variables	
Normozoospermia:	Normal ejaculates as defined above
Oligozoospermia:	Sperm concentration fewer than 20 X 10^6/ml
Asthenozoospermia:	Fewer than 50% spermatozoa with forward progression or fewer than 25% spermatozoa with rapid progression
Teratozoospermia:	Fewer than 30% spermatozoa with normal morphology
Oligoasthenotertozoospermia:	Disturbance of all the three variables (concentration, motility and morphology)
Azoospermia:	No spermatozoa in the ejaculate
Aspermia:	No ejaculate

Table 2: Seminal fluid analysis parameters (Nkposong, 1987) (20)

Minimal values compatible with fertility	
Volume:	2-5 mls
Viscosity:	Fully liquefied within 1 hour
Sperm count:	20 million/ml
Motility:	60% (1st hour), 50% (2-3 hours)
Motility index:	3 – 4
Morphology:	60% normal forms
Vital staining:	30% dead spermatozoa
Polymorphs:	5% (No significant pyospermia)
Immature forms:	5%

In addition to semen analysis providing diagnostic/predictive valùes for *in vivo* fertility and conception, a number of studies (21, 22) that have examined the predictive values of the traditional semen characteristics have shown these parameters to also predict pregnancy outcome during artificial reproductive techniques (ART).

Semen analyses results usually set the pace for further investigations of the infertile male. If the spermogram is normal in all respects, no further investigation is necessary. But up to 50% of males of infertile unions have been shown to have abnormal spermogram in Nigeria (23). These include those with oligozoospermia and azoospermia. In a male infertility clinic in Ibadan, Nigeria (19), 35% of the patients

attended to, had azoospermia while 48.9% and 23.4% of patients attending a male infertility clinic in Ilorin, Nigeria (20) had oligozoospermia and azoospermia respectively.

Hormonal evaluation of male infertility usually follows the observation of oligozoospermia or azoospermia. These patients will benefit from serum FSH, LH, PRL and testosterone determinations (25, 26). The commonest hormonal disorder in infertile Nigerian males is hyperprolactinaemia (12, 26) and it is associated with oligozoospermia and azoospermia (27). Up to 25% of infertile males Nigeria have primary testicular failure, probably due to infectious diseases that affect and destroy the testes (12). The common findings in such patients are severe oligozoospermia and azoospermia, elevated levels of serum FSH, LH and low serum testosterone.

Azoospermia and some cases of severe oligozoospermia can be either due to failure of spermatogenesis or obstruction disorder; intratesticular, epididymal, vasal or ejaculatory duct (19, 28). Serum FSH, LH and testosterone measurements are required in suspected cases of testicular failure or obstructive disorder (8). Determination of serum FSH can distinguish between primary and secondary gonadal failure, and can also identify those with obstructive azoospermia. Serum levels of FSH, LH, PRL and testosterone are usually normal in obstructive disorder Truly, serum FSH determination has reduced the need for testicular biopsy to. confirming normal spermatogenesis in cases of genital tract obstruction.

The histological findings in obstructive azoospermia include tubules with normal or slightly reduced diameter in the presence of all stages of spermatogenesis. Also, the normal orderly arrangement of testicular architecture is lost and the central lumen is absent. Half or more of the examined tubules must be so affected to make the diagnosis of obstructive azoospermia (29). Some of such patients suffer from Young's syndrome, in which the obstructive azoospermia and chronic sino-pulmonary infections co-exist (30). Testicular biopsy in non-obstructive azoospermia shows structural immaturity of the seminiferous tubules, decreased spermatogenesis, germ cell aplasia (Sertoli cell only syndrome), germ cell maturation arrest, peritubular and tubular fibrosis (30).

Another useful test that is advised in men with azoospermia and severe oligozoospermia is karyotyping, when chromosomal disorder is suspected (32). This is particularly important in the face of elevated FSH level and markedly reduced testicular volume. The most frequent chromosomal abnormalities in infertile males are sex chromosome aneuploidies; such as 47, XYY karyotypes, autosomal Robertsonian translocations and other types of translocation (33, 34).

Klinefelter's syndrome is the best known karyotypic abnormality and it is associated with a characteristic histologic appearance of the tissue (35). Even after puberty in Klinefelters', the histological findings could be that of no spermatogenesis or some degree of spermatogenesis. The finding of some amount of spermatogenesis should not militate against the diagnosis of Klinefelter's syndrome (36). Occasionally,

the classic histologic appearance of Klinefelter's syndrome occurs in the absence of karyotypic abnormalities (36).

Other congenital problems in infertile males include congenital bilateral absence of vas deferens (CBAVD) and congenital adrenal hyperplasia (CAH) (9). Patients with CBAVD will present as cases of obstructive azoospermia (37) while those with CAH have spermatogenic defect (9). Measurement of seminal alpha-glucosidase enzyme can serve as a non-invasive method of distinguishing obstructive from non-obstructive azoospermia (37). To help in making a diagnosis of CAH, serum level of 17-ketosteroids or dehydroepiandrosterone (DHEA) should be measured (9). Additional tests in these patients include seminal total antioxidant capacity (TAC) which is low in up to 40% of infertile males, due to increased generation of reactive oxygen species (38, 39).

ROLE IN TREATMENT

Effective treatment of male infertility begins with a careful history and physical examination. Specific childhood diseases such as cryptochordism, post-pubertal mump orchitis and testicular trauma/torsion should be sought. History of exposure to occupational and environmental toxins, excessive heat and radiation should be elicited. Drug history is equally important and such drugs as anabolic steroids, cimetidine and spironolactone, are known to affect reproductive cycle (18). Excessive alcohol consumption is associated with a decrease in sperm count and hormonal abnormalities (18). Past history of improperly treated sexually transmitted infections should be excluded in our environment.

Treatment could be medical or surgical or both. The role of clinical pathologists in the medical management of infertile males is prominent in those due to infections, either latent or ongoing. It is important that in such cases, the organisms associated with genital infections are accurately identified and treated with appropriate antimicrobial agents as well as anti-inflammatory drugs. Success of treatment can be monitored by periodic seminal fluid analysis.

However, more often than not, patients are seen at late stage when only commensal flora rather than genital pathogens are isolated (39). Nevertheless, attempt at identifying and treating any recent or latent infections should be done. In some cases, androgen may be administered to improve sperm count in those with mild to moderate oligozoospermia. Other medical treatment modalities include endocrine therapy for men with hypogonadotropic hypogonadism, immunosuppressive drugs (corticosteroids) for immunologic infertility and α-adrenergic stimulation using sympathomimetic drugs for those with retrograde ejaculation (18).

The commonly treatable surgical conditions associated with male infertility are undertaken by urologists. The commonest surgical procedure carried out on infertile male is varicocelectomy, because scrotal varicocoele is found in about 40% of infertile male (20). Surgical procedure can also be performed for cases of obstruction of the reproductive tracts when the spermatogenic potential of the testes is preserved, and pituitary adenoma can be removed by surgical ablation (18). The role of the clinical pathologists and the

laboratory in this regard include post-operative serial monitoring of patients for response to surgery. The concerned laboratory should ensure appropriate quality control measures so that subtle changes in seminal, endocrine and other laboratory indices are noted.

It should be emphasized that infertile males can benefit from assisted reproductive techniques (ART), to fertilize the ovum of their spouse. This is possible in those, in whom spermatozoa can be obtained by microsurgical epididymal sperm aspiration (MESA) following stimulation of spermatogenesis, or retrieval of spermatozoa from the urinary bladder in those with retrograde ejaculation (18). The clinical pathologist has a role to play in determining the semen parameters that are important in predicting pregnancy outcome or success rate of ART procedures in infertile males and their spouse.

THE NIGERIAN SITUATION

The high prevalence of azoospermia in Nigeria is worrisome. These are related possibly to infections (12, 20, 28, 40-44). With the emergence of new and increase in the number of antibiotic resistant venereal pathogens (43, 44, 45), complications from simple genital infection have increased with a consequent increase in the number of males with primary or secondary infertility (46, 47).

Hence, the quality of care offered at sexually transmitted diseases (STD) and infertility clinics need to be improved. Highly sensitive and specific newer diagnostic methods, such as the DNA probes and polymerase chain reaction (PCR) as well as serologic tests that can promptly detect acute and subclinical infections are now available (48) though not yet widely applicable in developing countries.

Prompt diagnosis and treatment of cases of STD is the backbone of prevention of male infertility in this environment. Contact tracing and treatment, community awareness campaign about the attendant sequelae of improperly treated STDs, health seeking behaviour and proper administration of the syndromic approach to STD management are valuable aspects of the control of STD related male infertility in developing countries.

Males who are infertile should also readily have hormonal evaluation especially serum PRL, FSH and LH, when they have abnormal spermogram. Clinical pathologists in our environment should as part of the assessment for laboratory investigation of infertile males, suspect common cause such as varicocoele which is associated with abnormal spermogram. This will require prompt referral to the urologist for appropriate treatment. Karyotypic studies are not readily available in most centres. This should be a matter for attention by pathologists in Nigeria.

CONCLUSION

In the management of male infertility, a team of clinicians and pathologists working closely together will enhance the achievement of the desired success in the diagnosis, treatment and prevention of this condition.

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