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MOLECULAR EPIDEMIOLOGY OF HEpatitis C VIRUS (HCV) IN KADUNA STATE.


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ABSTRACT

Objective: To determine the distribution of hepatitis C virus (HCV) genotypes and subtypes among blood donors and outpatients attendees positive for antibody to HCV (anti-HCV).

Justification: Hepatitis C virus (HCV) continues to be a major disease burden on the world and Man is the only known natural host of Hepatitis C virus (Chivaliez and Pawlotsky, 2007). There is no published data on the prevalence of the genotypes and subtypes of HCV in Kaduna State.

Setting: Three hospitals one in each of the 3 senatorial zones in Kaduna State.

Patients: Blood donors who reported for blood donation and outpatient department attendees.

Method: Antibody detection by a third generation HCV ELISA (Biotech Laboratories, UK); HCV RNA and genotyping by Reverse Transcriptase polymerase chain reaction with genotype-specific primers. (Sacace Biotechnologies, UK).

Results: of the 259 plasma specimens screened for hepatitis C virus in this study, 20(7.7%) were positive for anti-HCV antibodies by ELISA and 16(6.2%) of the antibodies positive specimen were positive for HCV RNA. Of the 139 blood donors tested, 8 (5.8%) were HCV RNA positive. Similarly, 120 were tested from the outpatient Department attendees and 8 (6.7%) were HCV RNA positive. Hepatitis C virus genotype 1b was found in the entire HCV RNA positive sample.

Conclusions: The findings of 6.2% prevalence of HCV infection based on HCV RNA test confirmed that there is Hepatitis C virus in Kaduna State with genotype 1b as the predominant genotype found in all the three senatorial zones.

INTRODUCTION

Blood test were developed to identify hepatitis B in 1963 and hepatitis A in 1973, but many of the blood samples taken for post transfusion illness tested negative for hepatitis A and Hepatitis B. The unidentified cases were classified as non A, non B hepatitis (1, 2). In the 1980's, investigators from the centre for disease and control and Chiron Corporation identified the virus to be Hepatitis C virus (2). In 1990, blood banks began screening blood donors for hepatitis C, and it was not until 1992 that a blood test was perfected that effectively eliminated HCV from the blood transfusion supply (2, 3). It is now believed that approximately 90 to 95% of cases previously classified as non A, non B hepatitis were actually hepatitis C (3). Hepatitis C virus (HCV) is a member of the family Flaviviridae, placed in a new monotypic genus-Hepacivirus (4, 5). The viral genome is a single-stranded RNA molecule approximately 9.6 kb in length which is positive sense and possesses a unique open reading frame, coding for a single polyprotein, flanked by untranslated regions at both its 5’ and 3’ ends. The length of the polyprotein-encoding region varies according to the isolate and genotype of the virus from 3008 to 3037 amino acids (6). The genus Hepacivirus consists of 6 major genotypes further divided into subtypes (7). HCV genotypes 1, 2 and 3 are the most commonly detected types worldwide (8). Genotype 1 in particular has been extensively reported by other authors in Brazil (9), Chile (10), Uruguay (11), Argentina (12) and Venezuela (13). HCV genotype 1a, 1b originated about 100 years ago and are evolving at faster rate than genotypes 4 and 6 (8).
transfusion-related HCV is however still higher in developing countries (15). Nigeria belongs to the group of countries highly endemic for viral hepatitis including HCV. The prevalence of HCV among blood donors ranges from 6.0% to 9.5% (16, 17, 18). However, there is no knowledge of HCV RNA prevalence in Kaduna state.

MATERIALS AND METHODS.
Ethical approval was obtained from the ethical committee of the Ahmadu Bello University Teaching Hospital Shika-Zaria and from the Directors of General Hospital Kafanchan and Yusuf Dantsoso Hospital Tudun Wada Kaduna. Informed consent form written in English and Hausa was administered to each person whose blood was collected. Two hundred and fifty nine (259) blood samples were collected from 139 blood donors and 120 outpatient department attendees in three hospitals one in each of the three senatorial zones in Kaduna state using systematic sampling method. Blood samples (5ml) were collected into blood bottles containing anticoagulant. The plasma samples were separated into sterile bottles by centrifugation at 1000 rpm for 30 minutes and the plasma samples were stored at -20°C initially in the various hospitals and were later transported by cold box and stored at -20°C in Virology laboratory at the Department of Microbiology, Ahmadu Bello University Zaria immediately until required for the analysis.

HCV ANTIBODY DETECTION BY HCV ELISA (BIOTEC U.K)
The plasma samples were initially tested for the presence of Hepatitis C virus antibodies using a commercially available third generation HCV Enzyme-linked immunosorbent assay (Biotech Laboratories, 1P53RG.UK.). The procedure according to the manufacturer was used. The plates were read according to manufacturer’s instruction as follows: The wells were assessed visually immediately after the second incubation. Medium to dark blue colour indicated positive result. No colour or very pale colour indicated negative result. The intensity of the reaction was photometrically quantitated with dual filter Enzyme Immuno Assay Reader (Sigma diagnostics ELIA Multifit Reader 11) immediately using O.D at 450nm, 630nm.

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR).
HCV RNA Extraction procedure.
The lysis solution and washing solution were brought to 65°C and Lysis solution (450µl) and 100µl of samples were added to appropriate labelled tubes. All tubes were vortexed and centrifuged for 30 sec. Sorbent was vortexed vigorously and 25µl were added to each tube and all tubes were vortexed for 7 sec and were incubated at room temperature for 10 minutes. The lysis solution and washing solution were brought to 65°C and Lysis solution (450µl) and 100µl of samples were added to appropriate labelled tubes. All tubes were vortexed and centrifuged for 30 sec. Sorbent was vortexed vigorously and 25µl were added to each tube and all tubes were vortexed for 7 sec and were incubated at room temperature for 10 minutes. The tubes were vortexed periodically during incubation. The tubes were centrifuged for 30 sec at 10,000(rpm) and the supernatants were discarded without disturbing the pellet using fresh pipette tips. Washing solution (400µl) was added to each tube vortexed vigorously and were centrifuged for 30 sec at 10,000rpm. The supernatants were then carefully removed without disturbing the pellet using fresh pipette tips. Seventy percent ethanol (500µl) was added to each tube and was vortexed vigorously and was centrifuged for 30 sec at 10,000rpm. The supernatants were carefully removed without disturbing the pellet with fresh tips between tubes. All tubes were incubated with open cap for 10minutes at 60°C. The pellets were re-suspended in 50µl of RNA-diluents and were incubated for 10 minutes at 60°C and were vortexed periodically during incubation. The tubes were centrifuged for 1 minute at maximum speed of 16,000rpm. The supernatant which contain the RNA were carefully removed into sterile tubes ready for use.

Reverse Transcription
Preparation of Reaction Mix for 12 Reactions
RT-G-mix-1 (5µl) was added into tube containing RT-Mix, vortexed for 10 sec and was briefly centrifuged. M-MLV (6µl) was added into tube with reaction mixed, vortexed and centrifuged for 7sec. This was immediately used for reverse transcription.

Reverse Transcription
Reaction mix (10µl) was added to each tube. The tubes with extracted RNA were re-centrifuged for 2 minutes at 16,000rpm. Supernatants containing extracted RNA (10µl) were taken to appropriate tubes. The tubes were placed into thermocycler(Techgene, model FTGENE 5D, Serial NO 121254-4) and were incubated at 37°C for 30 minutes. Each cDNA sample obtained was diluted 1:2 with T E-buffer [20µl of T E buffer was added to each tube] and was ready for used.

Enzymes and oligonucleotide primers for the polymerase chain reaction.
Taq deoxyribonucleic acid (DNA) polymerase and moloney murine leukemia virus (M-MLV) reverse transcriptase were obtained from (Sacace Biotechnologies, Italy). Primers utilized were specific for region of HCV genome with the 338bp for genotype 1a, 395bp for genotype 1b, 286bp for genotype 2, and 227bp for genotype 3a. Primers oligonucleotide sequences include:
5'-CAGTCACTGAGAGCGACATCCGTACG-3' (for 1a)
5'-AGGCCACTGCGGCCTGTCGAGCTGCGAA-3' (for 1b)
5'-TATGTTCAACAGCAAGGGCCAGA-3' (for 2).
5'-CTCGGACCCTGACTTTCT-3' (for 3a)
5'-CCTGGTCATAGCCTCCGTGAA-3' (antisense primer for all genotypes).

Polymerase Chain Reaction using HCV genotype specific primers.
Tubes (12) of PCR Mix-1 genotypes 1a/1b tubes and 12 of PCR Mix-1 genotypes 2/3a including 1 tube for negative control and 1 tube for positive control were prepared. PCR Mix-2 (10µl) was added to each tube. cDNA samples obtained after RT step (5µl) were added to appropriate tubes. DNA buffer (5µl) was added to negative control tube of amplification. cDNA 1a (5µl) was added to the PCR-Mix-1 genotype 1a/1b tube. cDNA 1b (5µl) were added to the PCR-Mix-1 genotype 1a/1b tube. cDNA 2 (5µl) were added to the PCR Mix-1 genotypes 2/3a tube. cDNA 3a (5µl) were added to the PCR Mix-1 genotypes 2/3a tube. The tubes were closed and transferred to the thermocycler only when temperature reached 95°C. The reactions were started after the thermocycler was programmed. Step 1 (Initial denaturation): 95°C, 5 minute. Step 2 (42 cycles): Denaturation at 95°C, 1 minute; annealing at 68°C, 1 minute; extension at 72°C, 1 minute. Step 3 final extension at 72°C, 1 minute and finally 10°C for storage temperature.

Detection of the PCR Products
The amplified cDNAs were detected by electrophoresis.
Agarose concentration of 1.0% was melted by boiling in microwave oven for 2 minutes. The solubilized agarose was cooled down to 65°C and 5µl ethidium bromide was added. Formoldehyde (6.5ml) was added to every 100ml of gel. The gel was poured into taped gel trays and well-formed combs were placed near the edge and middle of gel and were covered with plastic box to prevent evaporation and allowed to harden for 1 hour. Electrophoresis tanks were filled with 10mM sodium phosphate buffer. The gel was totally submerged in buffer at a level not more than 1cm above the gel and the combs were removed. Samples were prepared by adding 5µl of gel loading buffer to each 15µl sample. Samples were loaded and 75volts was applied for 45 minutes. The power supply was switch off when dye front has run approximately 80% of gel length and the gel trays containing the gel were removed. The gel were then soaked in 5mM NaoH and blotted into nylon membrane. Gels were examined on UV transluminator using protective glass. Photographs were taken and the resulting photographs were used to determine the distance migrated for each band.

Identification of Bands of PCR Products
The control cDNA for genotypes 1a, 1b, 2, 3a and kilobase ladder (used as marker) were used for the identification of the bands as shown in figure 1 below.
The length of specific amplified DNA fragments is:
HCV genotype 1a – 338 bp, HCV genotype 1b – 395 bp, HCV genotype 2 – 286 bp, HCV genotype 3a – 227 bp.

Fig. 1: Shows Agarose gel electrophoresis of HCV RNA using specific primers for genotype 1a, 1b, 2 and 3a. Analysis of the result is based on the presence or absence of specific bands of amplified DNA on agarose gel. Lane M, marker (1000bps); Lane 1, 3, 5, 7, 9, 11, 13, 15, 17 & 19, genotypes 1a/1b; Lane 2, 4, 6, 8, 10, 12, 14, 16, 18 & 20, genotypes 2/3a; Lane C1, positive control for genotypes 2/3a; Lane C2, positive control for genotypes 1a/1b; Lane CN, negative control of amplification.
DATA ANALYSIS
The data were analyzed at Data Processing Unit, Institute of Agriculture Research (IAR), ABU, Zaria using SPSS Software Version 13.0. Chi-square test was used at 95% confidence interval (P≤0.05).

RESULTS
Out of the 259 plasma specimens screened for Hepatitis C virus in this study, 20(7.7%) were positive for anti-HCV antibodies by ELISA and 16(6.2%) of the antibodies positive specimen were positive for HCV RNA (Table 1). The prevalence of HCV per age group has shown that 21-40 age group has the highest prevalence rate of 14(7.2%) HCV RNA followed by 41-60 age group with 2(5.6%) HCV RNA. Also, the P value (P = 0.735) indicated that there is no significance association between age and HCV infection. Of the 139 blood donors tested, 8 (5.8%) were HCV RNA positive. Similarly, 120 were tested from the outpatient Department Attendees and 8 (6.7%) were HCV RNA positive (Table 2).

Table 1: Distribution of HCV among blood donors and Outpatient Department attendees in the Senatorial Zones

<table>
<thead>
<tr>
<th>Zone</th>
<th>Number Tested</th>
<th>Number (%) Positive HCV Ab</th>
<th>HCV RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern</td>
<td>46</td>
<td>4(8.6)</td>
<td>4(8.6)</td>
</tr>
<tr>
<td>Central</td>
<td>130</td>
<td>12(9.2)</td>
<td>8(6.2)</td>
</tr>
<tr>
<td>Northern</td>
<td>83</td>
<td>4(4.8)</td>
<td>4(4.8)</td>
</tr>
<tr>
<td>Total</td>
<td>259</td>
<td>20(7.7)</td>
<td>16(6.2)</td>
</tr>
</tbody>
</table>

Chi square value = 0.286; P = 0.087 at 95% confidence interval (P≤0.05)

Table 2: Age Distribution of HCV Positive Cases

<table>
<thead>
<tr>
<th>Zone</th>
<th>Blood Donors</th>
<th>General Population</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) HCV Pos</td>
<td>No. (%) HCV POS</td>
<td>No. (%) HCV POS</td>
</tr>
<tr>
<td>≤ 20</td>
<td>4 (0.0)</td>
<td>24 (0.0)</td>
<td>28 (0.0)</td>
</tr>
<tr>
<td>21 – 40</td>
<td>113 (7.6)</td>
<td>81 (7.6)</td>
<td>194 (14.7)</td>
</tr>
<tr>
<td>41 – 60</td>
<td>22 (4.5)</td>
<td>14 (7.1)</td>
<td>36 (2.5)</td>
</tr>
<tr>
<td>61 – 80</td>
<td>0 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>139 (8.5)</td>
<td>8 (6.7)</td>
<td>259 (16.6)</td>
</tr>
</tbody>
</table>

Chi - Square Value = 2.006; P = 0.735 at 95% Confidence Interval P≤0.05

DISCUSSION
The prevalence rate of 7.7% anti-HCV antibodies found in this study indicated a high prevalence of the disease in Kaduna State. It is higher than the earlier report of 6.0% HCV prevalence from Jos (16), and slightly lower than the 8.4% HCV prevalence reported from Abuja (17). The RT-PCR finding of 6.2% HCV prevalence is slightly higher than the world Health Organization report of 5.3% HCV prevalence rate in Africa (19). The prevalence of 5.8% HCV infection among blood donors and 6.7% HCV prevalence among outpatient Department attendees has however shown that the outpatient Department attendees had the highest prevalence among the study group. This confirms that outpatient Department attendees are patients seeking medical attention while blood donors were assumed to be healthy individuals. Hepatitis C
virus genotypes were determined by RT-PCR with
HCV specific primers for genotype 1a, 1b, 2 and 3a and
primers for all other genotypes were also used.
Genetically, the HCV genotype 1b was found to be the
genotype in circulation in Kaduna State, while in
Abuja, genotype 1a and 1b had been reported (17).
Genotype assignment helps in assessing disease
prognosis and assist in establishing the appropriate
dosage and duration of treatment (3, 20). Genotype
assignment also helps in determining the type of
vaccine to be used. Currently there is no HCV vaccine.
The report of HCV genotype 1b in Kaduna State offers
valuable information for its consideration as a vac cine
candidate when the search for HCV vaccine seriously
on course becomes a reality.

CONCLUSION
The findings of 6.2% prevalence of HCV infection
based on HCV RNA test confirmed that there
is Hepatitis C virus in Kaduna State. Genotype 1b was
found in all the 16 positive HCV RNA samples. This
suggests that genotype 1b is the predominant
 genotype in Kaduna state.

RECOMMENDATION
Hepatitis C virus should be taken seriously and should
be included among the blood borne pathogen that are
tested before blood transfusion in government and
private hospitals in Kaduna state. The health system
should be strength to support all HCV infected
persons medically and socially, as well as supporting
vaccine development research.

ACKNOWLEDGEMENTS
We thank professor A .A. Amad for his high
contribution to this work. We appreciate the Director
of Centre for Biotechnology Research, Professor A. J.
Nok who allowed the used of the center. We thank the
entire staff of Biotechnology centre who assisted in one
way or the other. We thank the Kaduna State Ministry
of Health for financial support.

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A CRITICAL REVIEW ON HIV/AIDS AND WOUND CARE

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Abstract

Wound infections in AIDS patients increase discomfort, prolong hospital stay, render an additional burden upon an already debilitated patient and weaken the immune system further. Treatment must relate to the aetiology of the wound and take into account the patients underlying health problems. The treatment of wounds in HIV-AIDS patients is not different from the standard treatment. There are wound-related criteria for selecting the appropriate types of dressing. The best dressing for postoperative wound healing by secondary intention is unknown. Continuing wound evaluation and the appraisal of what dressing is useful for the type of wound and stage of healing is the basis of optimum wound care. Optimum wound care, emotional support; health education will enhance both the emotional and physical wellbeing of the HIV-AIDS patient.

Key words: Human immunodeficiency virus (HIV), Acquired immune deficiency syndrome (AIDS), wound infection, delayed wound healing, optimum wound care, dressing types, nutrition, and pain control

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is becoming an increasing problem to the general surgeon (1, 2, 3). It is caused by infection with the Human Immunodeficiency Virus (HIV) which is an RNA virus that infects predominantly human T lymphocytes. It is transmitted by contaminated body fluids and, after a variable latent period of up to 2 years, it produces diminished immunological function which is manifest as AIDS. The suppressed cellular immunity allows the development of malignancies (Kaposi’s sarcoma and lymphoma) and opportunistic infections including Pneumocystis carinii pneumonia, cryptosporidium, cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV) and fungi (candida) (4).

AIDS is a worldwide pandemic with the highest prevalence in sub-Saharan Africa. Since its discovery in 1981, AIDS has rivalled the worst epidemics in history. As of 2004 an estimated 25 million people have died, and 40 million are living with HIV. There are about 58000 HIV/AIDS patients in the UK. The incidence has apparently levelled in the USA and Western Europe and the mortality from these infections has decreased as highly active antiretroviral therapy (HAART) has become widely available (4, 5, 6, 7).

HIV-AIDS patients being immunosuppressed will suffer from wound healing impairment and an increased susceptibility to wound infection. It appears that patients who are HIV positive without AIDS have no increased risk of wound problems, while those with AIDS are more likely to have delayed wound healing (5, 6). Surgery should be offered to HIV-positive patients without AIDS based on standard indications just as for HIV negative patients, but AIDS patients with more advanced disease, low CD4 counts (< 100) or poor performance status are at an increased risk for poor wound healing (5).

Although surgical procedures can be safe and effective therapeutic modalities, the benefits of resolution of symptoms must be balanced against this risk. Aggressive surgical interventions must be undertaken with caution (7, 8). The consequences of wound infection in AIDS patients are grave. They increase discomfort, prolong hospital stay, render an additional burden upon an already debilitated patient and weaken the immune system further. A trial into ‘task-shifting’ from doctors to nurses in the management of HIV-infected patients receiving antiretroviral therapy has proved a success (9). It also would seem to be true with their wound care, as wound care has always been the main domain in surgical nursing, facilitated by the closer nurse-patient relationship.

Wound care

Wound healing is a complex but highly integrated process that involves several cell types. The ultimate aims of tissue repair are rapid restoration of tissue
continuity and a rapid return to normal function (10). Wound healing has three phases; a lag phase of 2-3 days which is the acute inflammatory response to injury; an incremental phase which involves the progressive collagen synthesis by fibroblasts and gain in tensile strength; and a plateau (remodelling) phase in which excessive collagen is removed with decrease in fibroblasts and inflammatory cells (11). The first 7-10 days is usually sufficient to allow removal of skin sutures without wound disruption (10). Factors that adversely affect healing need to be common knowledge to surgeons so that where possible they can be eliminated to enhance patient recovery and the cost-effectiveness of care. The characteristics required of a wound for rapid and sound healing are; no (or minimal) foreign material, no infection, accurate apposition, no excess tension, good blood supply and no haematoma separating edges (12). In a heavily contaminated or infected wound it may be appropriate to use delayed primary closure or allow healing to proceed by second intention (13). The World Health Organization suggestions for reduction of wound infection (14, 15) are as follows: 1) short preoperative stay to prevent acquisition of hospital acquired infection, 2) antisepsic shower before operation, 3) shaving kept to a minimum, 4) avoid wound contamination, 5) meticulous attention to surgical technique, and 6) as speedy an operation as it is safe. Wound care for HIV-AIDS patients should incorporate these suggestions although recent trials have shown no clinical benefit of antisepsic showering before operation in normal patients (16, 17).

Sources of infection in HIV-AIDS patients
The sources of infection are a) endogenous and b) exogenous (18). Endogenous infections arise from the patient’s normal bacterial flora which contaminate the wound and cause infection. Prevention is by scrupulous patient hygiene, using clean bed linen and clothing, and avoiding wound contamination by poor dressing technique (15, 19). Exogenous infections arises from cross infection through hospital staff (hands), other patients, equipment and the environment (e.g. dust on curtain rails, bed frames). Prevention is by washing hands using liquid soap and drying carefully after every patient contact. It is advisable to use a bactericidal agent prior to aseptic procedures including wound dressing. An alternative is alcoholic hand rub especially at the bedside. Infection control also includes the use of sterile gloves, forceps and hand washing after removal of gloves (14, 20). The principles of wound care in AIDS patients are little different from the standard treatments (1, 2, 6, 7).

Principles of wound care:
1. Aseptic technique; the use of sterilised instruments with disinfected hands in sterilised gloves working on an antiseptically disinfected wound and surrounding skin covered with sterilised drapes. Antiseptically prepared skin eliminates exogenous skin organisms which are an important source of post operative wound infection (21).
2. The type of wound will determine the approach. Is it surgical or non-surgical? If surgical, is it a clean wound? e.g. hernia repair; clean-contaminated? e.g. cholecystectomy; contaminated wound? e.g. colonic resection, or a dirty wound? e.g. peritonitis secondary to intestinal/abscess perforation. Is the wound superficial or deep? Non-surgical wounds may be traumatic laceration, degloving, pre-tibial injuries or burn injuries. All non-surgical wounds should be regarded as potentially contaminated (22, 23).
3. The choice of dressing depends on individual wound evaluation. Is it necrotic, sloughy, infected, and granulating or epitheliaizing? Is it flat or a cavity? Ideally the wound should be kept warm and moist, protected from infection and physical damage. Excessive moisture in the wound bed may soften and weaken the wound edges (maceration). As a result the wound may enlarge and pathogenic organisms will be able to penetrate the surrounding skin and cause infection (24). Exudates and dead tissue should be removed following copious saline irrigation especially of dirty wounds.
4. Continuing evaluation of the wound is essential to detect early tissue degeneration which will suggest a change in dressing, cleanser or debrider. Evaluating the old dressing is helpful (25, 26). Good blood supply to the wound may be promoted by refreshing the wound and healthy tissue maintained in and around the wound.
5. Wound sutures should be given careful consideration. As wound healing may be delayed, each individual suture line must be evaluated to establish if healing is complete (10). The timing of suture removal should not depend on dogma. It should follow the evaluation of the individual’s wound as there are various factors, especially the site and the patient’s general health including immune status which may delay healing. The loose sutures used to close the upper or lower eyelid wounds are often removed at 48 h. Elsewhere on the face, sutures are removed at between 4 and 5 days all because of their excellent blood supply. Where movement increases the risk of dehiscence, in hand and limb wounds for example, it is common to leave in sutures for 10 days. Sutures left in longer than necessary may encourage infection, and will leave unnecessary marks or keloids especially on negroid skin.
6. Wound drainage: closed suction drainage minimises the risk of wound infection. The drain should be removed as soon as the fluid ceases (17, 18).

Wound assessment
This has had a tendency to be broad, subjective, inadequate and providing inaccurate information. The practitioner should appreciate the stage of healing, the type of tissue and expectation of time to complete healing. The choice of dressing is commonly based on the type of tissue present or as a colour scheme, e.g. black, yellow, red, and pink. There are dangers with this type of classification, as ‘black’ tissue is usually necrotic, but it could also be
The patient should be able to continue with her
maximize compliance
control
chemically and to promote autolysis (27). 3) to further damage, 2) to debride mechanically, a grossly contaminated or recently debrided infected there is no substitute for frequent dressing change in reflect this.

at all times for normal cellular physiology. Desiccated need to be changed less often for a clean wound but tissue is dead tissue and must be sharply debrided. Promotion of epithelialization. These factors can then include debridement, promotion of granulation and factors that are important in promoting healing include the optimum environment for the wound, which should be: moist with exudates but not macerated; free of clinical infection and excessive slough; free of toxic chemicals, particles or fibres released by the dressing; at the optimum temperature; undisturbed by frequent or unnecessary dressing changes and at the correct pH. Research in this area continues in attempting to identify how this optimum environment can be achieved (24, 26). There are several reasons for using a dressing: 1) to protect from drying, infection, further damage, 2) to debride—mechanically, chemically and to promote autolysis (27). 3) to control—bleeding, exudate, pain, odour, 4) to maximize compliance—patient, nurse, doctor system. The patient should be able to continue with her normal daily routine and the dressing regime should reflect this. 5) to stimulate healing—pharmacologically, physically (12, 28, 29). Dressings need to be changed less often for a clean wound but there is no substitute for frequent dressing changes in a grossly contaminated or recently debrided infected wound. Wounds should be kept moist (but not wet) at all times for normal cellular physiology. Desiccated tissue is dead tissue and must be sharply debrided. Factors that are important in promoting healing include debridement, promotion of granulation and promotion of epithelialization. These factors can then be compared with the properties of the various dressings’ materials available. If a number of dressings satisfy the wound’s needs, the final choice is made by considering relative efficacy, cost, convenience and patient acceptability. There are a number of factors which influence the process of selecting the most appropriate dressing. These include the individual patient, the wound, and the type of dressing.

Dressing types

Modern wound management offer a whole host of materials and dressings that provides a variety of benefits in the process of wound healing (30). A range of modern dressings might consist of contact materials: gauze (not as first option, if at all), low! non—adherent dressings; semi—permeable films; hydrogels; hydrocolloids; alginites; foams; antimicrobials plus growth factors. The best dressing for postoperative wounds healing by secondary intention is unknown because of the small, poor quality trials existing which render the evidence for best dressing insufficient (30). Foam is best studied as an alternative to gauze and appears to be preferable in terms of pain reduction, patient satisfaction and nursing time (31, 32). The traditional dressings include gauze and ‘Camgau’, which are cheap but which have a tendency to stick to wounds causing trauma and pain on removal. Low adherent and non-adherent dressings provide a surface which is less traumatic to remove. Tulle dressings, if used correctly can be of low adherence. They are also cheap but their use is limited on wounds with high or viscous exudates. Semi permeable film dressings are made from a clear polyurethane film coated with an adhesive. They conform well, are resistant to shearing and tearing, and permit constant observation. They provide and maintain an environment conducive to healing. Such dressings are not suitable in the case of heavily exuding wounds (33, 34, 35). The main advantages of using a semi permeable film are: acts as a bacterial barrier, elastic and durable, permeable to water and gases. The main disadvantages are: tends to collect fluid, which can cause maceration, some are difficult to apply correctly, can only be use for a flat surface, and skin reactions can be caused by adhesive. Examples are Bioclusive, Cutfilm, Epiview Opsite Flexigrid, Tegaderm.

Hydrocolloids e.g. granuflex consist of a mixture of carboxymethylcellulose, pectins, gelatins and elastomers. They are available in pastes, granules and wafers. On contact with wound secretions, the hydrocolloid material forms a gel, providing the optimum conditions for moist wound healing (34). Patients and users should be warned that the gel becomes pus-like in appearance and they should expect to detect an odour. The correct size of dressing may be left in place for 5-7 days. The main advantages of using hydrocolloid dressings are: easy to use, durable, positive effect on healing and cost-effective. The main disadvantages are: they can cause an odour, sometimes difficult to remove, cannot be used for cavities and may cause over-granulation. Hydrofibre consists of sodium carboxymethylcellulose spun into a fibre. It is highly absorbent and retains exudates. The fibres ‘gel’ into a sheet. The dressing is indicated for moderate to highly exuding wounds. It can be used for infected wounds, as long as it’s changed daily. The main advantages are: they are comfortable, do not cause maceration of surrounding skin highly absorbent and can be used on sloughy wounds (33, 24). Its main disadvantages are: some forms are costly and appearance may be confused with alginate dressings e.g. aquacel.
Hydrogels e.g. *intralite* gel consist of insoluble polymers, water and propylene glycol. They interact with fluid and absorb and retain large volumes of exudates (36). These dressings are available in different forms- sheets or gels. They are effective debriding/ desloughing agents and may be applied throughout the healing cycle (27). A hydrogel dressing is used on necrotic tissue because it facilitates autolytic debridement of rehydrating dead tissue and promotes phagocytic and enzymatic activity. Their use in the acute surgical cavity wound is not yet reported and maceration can occur in wounds that are heavily exuding. Their main advantages are: comfort, absorbent and debriding properties and as potential carriers for other treatments. Their main disadvantages include the requirement for frequent changing, can cause maceration of surrounding tissue, some forms are costly, and difficulty to retain on wound because of relative liquidity.

Alginate dressings e.g. *Sorbzan*, *Kaltostat* occur naturally as mixed salts of alginic acid and are found in and extracted from certain species of seaweed. When used on moderately or heavily exuding wounds, alginates will absorb secretions to form a gel, and this gel provides the optimum humidity and temperature conditions for moist wound healing (34). They are available in sheet form, packing/ribbon and as extra-absorbent sheets. Gentle irrigation with normal saline is effective in removing the dressing. Their main advantages are: varying absorbencies, forms a gel which is easily removed, can be used to pack sinuses and some have haemostatic properties. The main disadvantages are not to be used on dry wounds; sometimes require a quantity of saline to remove and require frequent changing if wet.

Foam dressings consist of polyurethane or silicone foam. They are absorbent (negative pressure wound dressings) and retain a moist environment (31). They are suitable for a wide range of granulating wounds, both flat and cavity. Wound healing for even chronic wounds can be greatly increased (30). Great prudence should be used; apply negative pressure wound dressing only when indicated. They are currently two types of cavity foam dressings: 1) liquid foam polymer- comes in two parts, a base and a catalyst, which are mixed directly to form a setting mixture. This sets to form a soft, spongy stent of the cavity. 2) Hydrocellular cavity wound dressing is available in a range of preformed sizes. Advantages of using foam dressings are: thermal insulation, protection (prevents 'strike through' of exudate to wound surface), permeable to water/gases, easily shaped and varying degrees of absorbency. The disadvantages are; costly and not always available. Examples are *Allevyn*, *Flexipore*, *Lyofoam*, *Spyrosorb* and *cavicare*.

![Image 1: Foam (Allevyn) dressing of a granulating laparotomy wound](image)

**Antimicrobials:**

Aseptic techniques help decrease the risk of infection as does the use of antibiotics in wounds that are contaminated (21). The use of topical agents has been challenged in the last decade, which has led to a decline in their usage. Antiseptics can be toxic when used on open wounds and disinfectants including hypochlorite solution such as Dakin’s solution, Edinburgh universal solution (Eusol) are also toxic to healing tissues but have anecdotally found a role in debridement and in wound bed preparation prior to skin grafting. However, given the problems surrounding the overuse of systemic antibiotics (rise in resistant organisms notably MRSA and emergent infections such as *Clostridium difficile*), many clinicians are now reviewing the clinical and experimental evidence against antiseptics with renewed enthusiasm (37). All wounds, regardless of type, may become colonised by microorganisms. However, the presence of bacteria does not necessarily indicate infection. It is the species of bacteria present or the rapid increase in a number of pathogenic bacteria past a certain critical level that leads to a clinical infection which is detrimental to wound healing.8 Clinical infection may necessitate treatment with systemic antibiotics and/or the use of a medicated dressing. These dressings include medicated tulle's, iodine (Inadine) and silver- based products (13). In neutropenic HIV/AIDS patients with disseminated candidiasis, wound swabs of chronic (delayed-healing) wounds will grow fungi that would respond to systemic antifungal medication (38,40).

HIV infected patients are at high risk for community-acquired MRSA. Monitoring the results of antimicrobial sensitivity profiles in their patient population will allow the clinician to base the empiric antimicrobial therapy upon local resistance patterns. Incision and drainage of a cutaneous abscess may be required and the wound should be cared for using standard wound care techniques. Subsequent follow-up is necessary to care for the healing wound and to assure that the empirical antimicrobial agent being utilized is likely to be effective based upon the
tissue' engineered skin can facilitate cell proliferation, grafting after early wound excision and removal of close large wounds especially burn wounds by skin invasion and proliferation. Therefore it is essential to increase local concentrations of growth factors in the production of extracellular matrix components and Dermograft, and composite grafts. i.e. those components, e.g. Vivoderm, dermal components, e.g. wound. Skin substitutes include epidermal colonisation of Artificial and living skin equivalents available and the application method. It may need to be considered in relation to the use of growth factors: the growth factor administered must be appropriate to the stage of healing; the wound should actually be deficient in the growth factor being applied; the growth factor should be delivered in sufficient amount and duration to produce the desired response. Examples are epidermal growth factor (EGF), transforming growth factor (alpha TGF alpha and betaTGFbeta); platelet derived growth factor (PDGF), fibroblast growth factor (FGF); and tumour necrosis factor (TNF). The disadvantages of using growth factors are: cost of treatment, treatment may not be available and the application method. It may need to be injected into the wound, and the choice of which growth factor, when, and what dosage is a problem (42). Growth factors are obviously not an alternative to good wound care.

Recent innovations

Growth factors

Developments in molecular biology have elucidated 30 biological mediators that are known to have an effect on wound healing. They play an important role in the normal wound healing process and have the potential to be used as a topical treatment for wounds to promote healing. In normal intact skin there is biochemical evidence of continual cytokine activity with a balance between inhibitory and stimulatory factors to provide a stable epidermis. In the presence of tissue injury, as with an acute wound, the growth factor activity increase. The following issues should be considered in relation to the use of growth factors: the growth factor administered must be appropriate to the stage of healing; the wound should actually be deficient in the growth factor being applied; the growth factor should be delivered in sufficient amount and duration to produce the desired response. Examples are epidermal growth factor (EGF), transforming growth factor (alpha TGF alpha and betaTGFbeta); platelet derived growth factor (PDGF), fibroblast growth factor (FGF); and tumour necrosis factor (TNF). The disadvantages of using growth factors are: cost of treatment, treatment may not be available and the application method. It may need to be injected into the wound, and the choice of which growth factor, when, and what dosage is a problem (42). Growth factors are obviously not an alternative to good wound care.

Artificial and living skin equivalents

Topical antimicrobials decrease the rate of colonisation of a wound, but do not prevent microbial invasion and proliferation. Therefore it is essential to close large wounds especially burn wounds by skin grafting after early wound excision and removal of toxic nonviable tissue. Depending on its composition, tissue-engineered skin can facilitate cell proliferation, production of extracellular matrix components and increase local concentrations of growth factors in the wound. Skin substitutes include epidermal components, e.g. Vivoderm, dermal components, e.g. Dermograft, and composite grafts. i.e. those containing epidermal and dermal components, e.g. Apligraf. No synthetic dermal replacement has been found to equal allograft dermis in closing wounds (43, 44).

Nutrition

Adequate nutrition aids the ability to cope with surgery and decreases the risk of post operative infection. It assists recovery (the body’s response to trauma), promote wound healing, immune function and avoids complications like deep vein thrombosis and pressure sores (45, 46). The assessment of nutritional status preoperatively identifies those at risk. Wound has high priority when competing with unwounded tissue for body resources. Therefore malnutrition has to be very severe before healing is affected. Wound dehiscence and infection are common when the serum albumin is low. When recent weight loss is greater than 20% of original weight healing problems can be anticipated (47). Protein meal and vitamin C (ascorbic acid) should be encouraged. The protein, collagen is the basic framework of a healed wound. It is synthesised within the endoplasmic reticulum of the fibroblasts and leaves the cell as tropocollagen. Proline and lysine are essential for collagen formation and oxygen and ascorbic acid (vitamin C) are necessary for proline hydroxylation and incorporation into tropocollagen. Tropocollagen polymersises between cells to form cross banded collagen fibrils (46, 48). Zinc is a component of enzymes involved in the healing process. Its deficiency retards healing. Supplements of ascorbic acid and zinc are effective when these factors are deficient but do not improve healing in normal subjects. Some patients may need nasogastric enteral feeding if the gut is functional. If insufficient; nutrients should be given as total parenteral nutrition (TPN) (48, 49). Over the past decade there has been increasing interest in the use of specially formulated feeds, both enteral and parenteral, to modulate the immune response to injury and illness. Although no study has specifically examined the role of these feeds in HIV/AIDS patients, there is increasing evidence that they may be beneficial in the management of malnourished surgical and critically unwell patients (48, 50). Glutamine is considered to be a 'conditionally essential amino acid; although it is synthesised in most tissues, plasma concentrations become depleted when consumption is increased, such as in metabolic stress and infection. Glutamine acts as both a nitrogen and energy source for lymphocytes and small intestinal mucosa, and reduced concentrations are associated with immune dysfunction and poor outcomes (51).

Patient comfort

Pain control is essential in reducing complications postoperatively. Pain causes a rise in cortisone via the hypothalamo-pituitary axis. Cortisone causes gluconeogenesis and this delays wound healing as protein is depleted. Increased cortisone also dampens immune function. Strong analgesics (e.g. opiates)
However, should be avoided as they can depress respiration and predispose chest infection. For painful short procedures for example wound dressing and physiotherapy entenox (nitrous oxide gas) may be given (3, 52).

**Summary**

The treatment of wounds in HIV-AIDS patients is not different from the standard treatments. The treatment must relate to the aetiology of the wound and take into account the patient’s underlying health problems (including medical history, medication, nutritional status, lifestyle e.g. tobacco and alcohol habits, psychological problems and quality of life). Prior to selecting the best dressing the characteristics of the wound bed such as necrotic tissue, granulation tissue, infection; the condition of the surrounding skin (normal, oedematous, white, shiny, warm, red, dry, scaling, thin) and the clinical signs of critical colonisation or local infection: delayed healing, odour, abnormal granulation tissue, increased wound pain and excessive exudate, are evaluated. There are wound-related criteria for selecting the appropriate types of dressing. An ideal dressing should maintain a moist environment for healing, enable trauma-free removal, facilitate gaseous exchange, impermeable to organisms and free from particulate or toxic contaminants. However, the best dressing for post operative wounds healing by secondary intention is unknown. Successful wound management depends on a flexible approach to the selection and use of products based on the understanding of the healing process and a knowledge of the properties of the various dressings available. For example the following choices are a suitable treatment for removal of necrotic tissue; surgical debridement, use of hydrogel dressing, use of hydrocolloids, use of an enzymatic agent, and use of occlusive dressing. Exposure to air will only serve to dry the necrotic tissue more and not allow the moist environment needed for cellular activity. Necrotic tissue requires either moisture in some form or sharp debridement. An infected wound may require surgical revision to facilitate free drainage. An alginate dressing that facilitates haemostasis and absorbs exudate would be appropriate at this stage. More traditional dressings such as gauze packing are difficult to remove and are extremely painful for the patient. Granulating and epithelializing wounds will require protection from further trauma. Dressings that are suitable are alginates, foams or hydrocolloids.

The aim of cavity wound management is to lightly pack the cavity to maintain free drainage. Absorption of exudate and ease of removal would be the two main considerations. Alginate and cavity foam dressings are the usual treatment of choice as they are easier to use in terms of application. Film dressings are indicated for superficial wounds and will not absorb exudate. The use of medicated dressings should be reserved for wound infection. There have been drastically improved surgical outcome as compared to the past with the advent of HAART, supported by the preventive measures against occupational HIV transmission, good anaesthetic and preoperative care. The AIDS-related surgical emergencies such as gut (Non-Hodgkin’s) lymphoma, infective colitis (bacterial CMV) kaposi sarcoma have declined. With increasing survival of these patients, however, the other non-AIDS related elective or emergency surgery predominate and thus wound care will still be needed for these infected patients.

**Conclusion**

HIV-AIDS patients should not be discriminated against with regard to receiving appropriate surgical intervention for fear of wound healing problems. Surgical procedures can be safe and effective therapeutic modalities. There are minimal problems with wound healing if wound care for HIV-AIDS patients incorporate the WHO suggestions or the UK NICE guidelines for reduction of wound infection. Continuing wound evaluation and the appraisal of what dressing is useful for the type of wound and stage of healing is the basis of optimum wound care. Adequate nutrition especially in HIV/AIDS patients facilitates wound healing and decrease wound infection by improving immune function. The improved surgical outcomes together with the preventive measures against occupational HIV transmission have resulted in the treatment of HIV/AIDS patients becoming an accepted part of routine surgical practice. Minimally invasive surgery is particularly relevant to both the HIV/AIDS patient and the surgeon, as in the former the adverse effect of wound healing and surgical outcome, and in the latter the risks of exposure are both minimised. Optimum wound care, emotional support, health education will enhance both the emotional and physical well-being of the HIV-AIDS patients.

**References**

patients in the era of highly active antiretroviral therapy. Archives of Surgery 141:1238-1245
THE SERO-PREVALENCE OF PARVOVIRUS ANTIBODIES AMONG CHILDREN WITH SICKLE CELL ANAEMIA IN ZARIA

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ABSTRACT
Parvovirus is an erythrovirus that infects red cell precursors in individuals with conditions characterised by a high red cell turnover like sickle cell anaemia and thalassaemia. Arthritis, vasculitis, carditis, bone marrow failure, and the slapped cheek appearance have been associated with Parvovirus B19 infection. Recurrent blood transfusion is a risk factor for the B19 serotype of Parvovirus infection, with the P antigen as the mediator for erythroid invasion presenting as transient erythroblastopenia (TEB). Although TEB is self-limiting a few cases may progress to aplastic anaemia. Previous studies report seroprevalence rates of between 44 and 71%, but the dearth of data on the seroprevalence of B19 parvovirus strain in our region prompted this study.

Venous blood samples from 239 children aged 1 to 15 years of consenting parents and guardians were screened for Parvovirus B19 IgG antibodies using the ELISA technique and antibody titre assessed spectrophotometrically. All the participants have sickle cell anaemia, but were in the steady state. Of this serum samples from 204 (85.4%) participants were positive for IgG antibodies against Parvovirus B19 while 35 (14.6%) were negative for the IgG antibodies. The age-group with the highest prevalence is 10-12 year group with seroprevalence rate of 88.9%. The overall seroprevalence of Parvovirus B19 antibodies is 85.4%.

The seroprevalence of Parvovirus B19 antibodies is high in all socio-economic groups. Antibody prevalence is higher in the non-transfused group suggesting that other factors than transfusion play a role in the spread of the B19 strain of Parvovirus B19.

INTRODUCTION
Parvovirus is of the family paroviridae, sub family parovirinae; which infect vertebrates as well as the subfamily densovirinae which infect invertebrates. (1,2). Members of Parovirinae are Amdovirus, Bocavirus, Dependovirus, Erythrovirus, Parvovirus, Partetavirus and Parvovirus(1). Parvoviruses are erythroviruses in view of their ability to replicate independently in erythroid and megakaryocyte precursors within the bone marrow (2). Parvoviruses also infect endothelial and myocardial cells(2). While Parvovirus is selectively pathogenic to humans, other parvovirus strains are Simian, Pig-tailed macaque, and Rhesus parvoviruses depending on the nature of their hosts (2).

Parvovirus is a non-enveloped isometric virus with a diameter of 18-26nm. Crystallography at 3.5 Å determined that the parvovirus is an icosahedral virus with a polypeptide fold of 2 to 3 major capsid proteins, namely VP1, VP2 and VP3 (1,3). The genomic particle consists of 60 copies of capsid protein containing single stranded Deoxyribonucleic acid (DNA). Parvovirus genome contains 5596 nucleotides, of this 4830 nucleotide sequences constitute the encoding sequences; and 383 flanking sequences on both sides form the inverted terminal repetitive sequences(2).

The parvovirus B19 also possesses two non-structural proteins NS1 and NS2 which bear some homology with polyoma, papilloma and Porcine parvovirus (PPV) viruses. Parvovirus’ tropism for erythroid progenitor cells is the basis aplastic crisis in persons with chronic haemolytic states like sickle cell anaemia, hereditary spherocytosis, and thalassaemia (5-8).

There are three genotypes of the Parvovirus namely B19, LaLi, and V9, but the B19 serotype is the most significant having been implicated in acute febrile illnesses of childhood like erythema-infectiosum or the slapped cheek or fifth disease indicating cytopathic effect on endothelial cells. The cellular receptor for Parvovirus is the P antigen which is present on endothelial and myocardial cells as well as precursors of erythroid and megakaryocyte lines.

Cytopathic effect following viral replication within infected erythroid precursors results in giant cell formation. The virus is spread by droplet infection through the naso-pharynx; recurrent blood transfusion and immunosuppression are also risk factors for infection(8). Foetal loss results from foetal red destruction in non-immune pregnant women (6-7). Arthritis, vasculitis, myocarditis, liver and bone marrow failure are additional clinical outcomes of parvovirus infection. Sickle cell individuals are prone to acute splenic sequestration, meningo-encephalitis, acute chest and cerebral syndromes. Diagnosis depends on demonstration of...
Immunoglobulin M (IgM) antibodies within 1-2 weeks of infection and Immunoglobulin G (IgG) afterwards. Demonstration of viral nuclear components by polymerase chain reaction is the confirmatory test. But shared homology with some viruses like the porcine parvovirus poses a challenge in specificity when PCR is the basis of diagnosis, therefore real-time PCR is an alternative when cross-reactivity is suspected. (1-4N) Bone marrow involvement resulting from viraemia causes selective destruction of red cell precursors with a varying natural history, this ranges from a self-limiting transient erythroblastopaenia (TEB) and reticulocytopenia; to a chronic transfusion dependent condition characterised by bone marrow aplasia (5, 7).

The prevalence of parvovirus antibodies ranges from 44 to 71% with an overall prevalence of 53% in one study. The team from the children’s hospital of Philadelphia reported an IgG prevalence rate of 70%. The paucity of data on the prevalence of Parvovirus B19 infection despite reported cases of transfusion dependence in persons in this environment prompted this study.

MATERIALS AND METHODS
Two hundred and thirty nine children aged 1 to 15 years with sickle cell anaemia who attend the sickle cell clinics of Ahmadu Bello University Teaching Hospital, Shika Zaria and Barau Dikko Specialist Hospital, Kaduna but were in the steady state were the participants in this cross-sectional study. Consent was obtained from their parents and guardians. Their serum samples were screened for Parvovirus B19 IgG antibodies using the Enzyme Linked Immunosorbent Assay (ELISA) technique applying kits manufactured by DRG instruments GmbH Frauenbergstrate 18, 35039 Marburg Germany.

ELISA Procedure for Anti-Parvovirus IgG Assay
Quality Control Measures
The kits are purchased in packs of 96 tests per kit.

Each test kit contains an internal quality control pack to validate the efficacy of the assay and results according to the manufacturer’s instructions.

Each test kit has 5 wells reserved for blanks and controls.

Low positive was tested in duplicate.

A well was left empty for the substrate blank which was mixed by gently tapping all sides on a flat surface.

ELISA method
About 100µl of conjugate was transferred to each well of the micro plate except the blank. Bubbles were avoided upon addition. It was then mixed well by gently tapping all microplates sides on a flat surface.

The plates were covered with an adhesive seal and incubated for 30 minutes at room temperature (30-32°C). The adhesive seal was removed and discarded. Wells were aspirated and washed.

About 100µl of substrate was added to each well including the blank.

After this, it was incubated for 15 minutes at room temperature and (30-32°C) and protected from light.

Finally, the reaction was stopped by adding 100µl of stop solution in each well including the blank, in the same sequence and time intervals as for the substrate addition. The plate was gently tapped to mix the content of the wells.

Plates were read according to manufacturer’s instructions as follows.

Dark precipitates of the chromogen to yellow colour indicated positive result
Pale yellow colour indicated negative result.

The intensity of the reaction was photometrically quantitated with a dual filter Enzyme Immuno Assay Reader (Sigma diagnostics EIA Multi-well Reader 11) after one hour using O.D. at 450nm, 630nm.

RESULTS
Of 239 participants, 120 were males and 119 were females. Serum samples from 204 (85.4%) out of 239 participants were positive for IgG antibodies against Parvovirus while 35 (14.6%) were negative for the IgG antibodies. Therefore, the seroprevalence of Parvovirus antibodies is 85.4 % (Table1). The seroprevalence rate by gender is 99(82.5%) and 105(88.2%) for males and females respectively but there is no statistically significant difference between the genders (Table2). The age-group with the highest prevalence is 10-12year group with seroprevalence rate of 88.9% (Figure1).

The prevalence rate for Parvovirus antibodies was more than 80% in all socio-economic groups and the difference in prevalence rates between the groups was not statistically significant with the children of persons in the higher socio-economic groups having highest prevalence rates. Fifty three (22.2%) were previously transfused and 186(77.8%) were not transfused. The sero-prevalence by transfusion is 43/53(81.1%) and 161/186(86.6%) for the transfused and non transfused group respectively. There is no statistically significant difference between the prevalence rates.
TABLE 1: PREVALENCE OF PARVOVIRUS B19 IG G AMONG CHILDREN WITH SICKLE CELL DISEASE

<table>
<thead>
<tr>
<th>Status</th>
<th>No. of children screened</th>
<th>Percentage positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>35</td>
<td>14.6</td>
</tr>
<tr>
<td>Positive</td>
<td>204</td>
<td>85.4</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>100%</td>
</tr>
</tbody>
</table>

X² = 0.001, P < 0.05 at 95% CI

There is a significant association between Immunoglobulin G antibodies to Parvovirus B19 and sickle cell disease.

TABLE 2: DISTRIBUTION OF PARVOVIRUS B19 IG G AMONG THE DIFFERENT SEXES IN CHILDREN WITH SICKLE CELL DISEASE

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of screen samples</th>
<th>No. of positive samples</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>120</td>
<td>99</td>
<td>82.5%</td>
</tr>
<tr>
<td>Female</td>
<td>119</td>
<td>105</td>
<td>88.2%</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>204</td>
<td>85.4%</td>
</tr>
</tbody>
</table>

X² = 0.210  95% CI

There is no significant association between the presence of IgG to Parvovirus B19 and gender.  P > 0.05

DISCUSSION

The majority formed by males in this study is contrary to the observations by Konotey Ahulu, Aliyu et al, and Mamman et al in which females were the majority (10-12). This may be explained by the fact Ahulu, Aliyu and Mamman conducted their studies on adult patients and females are more likely to comply with clinic attendance than their male counterparts. The prevalence rate of 85.4% obtained in this study is higher than 70%, 60% and 56.5% reported by Eis-Hüibinger, Dayana and Regaya et al. (13-15). The highest prevalence was in the 10 to 12 year age group. This is at variance with Ohene Frempong study in which the age 1 to 5 year group had the highest prevalence (9).

The proportion of participants who had a previous history of blood transfusion is 22.2% in this study. This is less than 60.8% reported by Mamman and Durosinmi (12). This suggests that transfusion demand increases with age as complications manifest. Paradoxically, the non-transfused group had a higher IgG antibody prevalence rate of 86.8% than 81.1% observed in the transfused group. Therefore, other factors than blood transfusion contribute to the transmission of Parvovirus.
FIGURE 1: THE BAR CHART SHOWS THE OVERALL AGE GROUP DISTRIBUTION OF PARVOVIRUS B19 IMMUNOGLOBULIN G AMONG CHILDREN WITH SICKLE CELL DISEASE IN SOME PARTS OF KADUNA STATE.

There is no significant association between the presence of Ig G to B19 virus and age group.

TABLE 3: THE DISTRIBUTION OF PARVOVIRUS B19 IGG AMONG CHILDREN WITH SICKLE CELL DISEASE OF DIFFERENT SOCIO-ECONOMIC STATUS BASED ON THEIR FATHERS’ OCCUPATION.

<table>
<thead>
<tr>
<th>Socioeconomic status / Fathers’ occupation</th>
<th>No. screened</th>
<th>No. positive</th>
<th>Percentage positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmers/Cottage craft/ Unemployed</td>
<td>125</td>
<td>107</td>
<td>85.6%</td>
</tr>
<tr>
<td>Business men/Traders/ Civil servants</td>
<td>95</td>
<td>80</td>
<td>84.2%</td>
</tr>
<tr>
<td>Professionals/Politicians</td>
<td>19</td>
<td>17</td>
<td>89.5%</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>204</td>
<td>85.4%</td>
</tr>
</tbody>
</table>

There is no statistically significant association between the presence of Parvovirus Ig G and socio-economic status.

The prevalence rate of 82.4% in the first three years of life suggests early infection probably due to droplet or aerosol spread (5, 6). The ubiquitous nature of the virus is indicated by near identical prevalence rates across all socio-economic groups as children of farmers, cottage craftsmen had a prevalence rate of 85.6% on the one hand while children of highly skilled professionals and politicians had a prevalence rate of 89.5% on the other.
TABLE 4: DISTRIBUTION OF PARVOVIRUS B19 IgG IN BLOOD TRANSFUSED AND NON BLOOD TRANSFUSED CHILDREN WITH SICKLE CELL DISEASE.

<table>
<thead>
<tr>
<th>Blood transfusion Status</th>
<th>No. children screened</th>
<th>No. positive</th>
<th>Percentage positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-transfused</td>
<td>186</td>
<td>161</td>
<td>86.6%</td>
</tr>
<tr>
<td>Transfused</td>
<td>53</td>
<td>43</td>
<td>81.1%</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>204</td>
<td>85.4%</td>
</tr>
</tbody>
</table>

$X^2 = 0.324$, $P > 0.05$, 95% Confidence Interval

There is no significant association between blood transfusion and Parvovirus B19.

Although IgG antibodies were detected by ELISA, this is limited by cross-reactivity with other parvoviruses, therefore Nucleic Acid Testing and PCR are confirmatory tests considering their sensitivity and specificity. Neither PCR nor NAT were carried out in this study due to logistic and infrastructural challenges in our settings in which Nigeria is 156th on the Human Development Index (16). In conclusion, this calls for studies that will assess the prevalence of IgM activity with the aim of detecting on-going infection, PCR based sero-epidemiology that will provide a basis for vaccine development.

REFERENCES

Evidence of long-term benefit of praziquantel treatment against Schistosoma mansoni in Kigungu fishing village of Entebbe, Uganda

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Abstract.

Praziquantel (PZQ) is efficacious against all species of schistosome: Schistosoma mansoni; Schistosoma haematobium; Schistosoma japonicum and other parasites like the Taenia species. This cross-sectional cohorts study was carried out in Kigungu fishing village along Lake Victoria shore in Entebbe Uganda. Our analysis was based on examining microscopically three slides from a single stool specimen from each of base line cohorts 945. These included children and adults, participants from both sexes in Kigungu fishing village in Entebbe Uganda. Nine hundred and one (901) of the cohort s were reexamined after six months and 625 of the same cohorts who were examined at the baseline and after six months were reexamined 18 months later. The slides were prepared using modified Kato/Katz (Odongo-Aginya) method. The infection proportion with Schistosoma mansoni at baseline was 448 (47.5%) but this was reduced to 244 (25.8%) 18 months after treatment with a single oral dose of praziquantel at 40mg/kg. However 495 (52.5%) were negative at the baseline study. The cure proportion after six months was significant (P=0.00), (OR4.63) CI at 95% (3.53-6.06). Similarly the cure proportion after 18 months was significant (P=0.00), (OR2.2) CI at 95% (1.87-3.34). The force of re-infection after six months was significant (P=0.0001), (OR 0.47) CI at 95% (0.31-0.71). Nevertheless the force of re-infection was not significant after 18 months (P=0.766), (OR 0.95) CI at 95% (0.68-1.34) eggs excretion did not reach the level of the pre-treatment intensity. The egg reduction was 69.3%. This was associated with age and pre-treatment intensity < 400 eggs per gram (egp) of faeces and age groups ≥ 30 years. The egg reduction also resulted in marked decrease in clinical symptoms in the participants. Our study suggests evidence of long-term benefit of praziquantel in Kigungu and that the re-infection occurred more commonly in younger age group than in the older patients.

Key words: Praziquantel; Schistosoma mansoni; Kigungu; Entebbe; Uganda.

Introduction

Praziquantel (PZQ) is efficacious against Schistosoma mansoni; Schistosoma haematobium; Schistosoma japonicum and other parasites like the Taenia species (1). Presently in Uganda, one of schistosomiasis endemic countries in sub-Saharan Africa, PZQ is the drug of choice in controlling morbidity due to schistosomiasis because the mean cost of treatment per dose per person in Uganda is $0.3 (1, 2, 3). In spite of its low cost, Uganda is still unable to procure adequate PZQ for mass chemotherapy (2). Consequently it was found necessary to establish a treatment regimen for revaluation study in Uganda. Previous studies in Uganda and elsewhere reevaluated the efficacy of PZQ after six months to establish the cure proportion (4, 5). Nevertheless other studies reevaluated the efficacy of PZQ at the interval of one year for a longer period (6, 7). At present, studies in different endemic settings using the single oral dose regimen of 40 (mg/kg wt) of PZQ against S. mansoni, S. haematobium and mixed infections, the efficacy of PZQ is reported to stand at 60-90 % (8). Extensive use of PZQ in Uganda and elsewhere in the tropics has been linked to the development of parasite resistance to the drug. This is evidenced by reduced cure proportion in humans treated with PZQ in the Richard Toll area of Senegal (3). However, factors like intensity of infections and high transmission of infections have been known to influence schistosomicidal activity of PZQ(4). Pharmacologically, Praziquantel kills adult worms by penetrating and damaging tegument of the adult worms (9). Subsequently there is a transient increase of antigens released from the dead adult worms and egg antigens in the circulation, which stimulate humoral and cellular immune responses of the host (10, 11). These anti-worm and anti-egg responses in the host aid in the killing of the worms by antibody binding to the antigen exposed due to Praziquantel damaged tegument (12). This phenomenon may continue for long after the pharmacological effect of...
Repeated treatment with PZQ has been shown to improve cure proportion as observed in West Nile districts of Uganda (13, 14). These are districts with high transmission and high intensity of infection (13, 15).

This study investigated the effect of PZQ on cure proportion and egg reduction 18 months after treatment in Kigungu-fishing village, a community with high exposure risk to S. mansoni infection in order to establish cost effective treatment regimen that could be adopted in Uganda schistosomiasis mansoni endemic foci (16).

MATERIALS AND METHODS

Study sample

A baseline evaluation study was carried out on a cohort of 945 volunteer adults and children who were residents of Kigungu fishing village, Entebbe, Uganda. Enrolled 448 patients were stool positive for S. mansoni while enrolled 495 patients were stool negative for S. mansoni. Treatment was administered to the 448 S. mansoni positive patients. Six months later 95.5% (901/945) patients were reviewed. These consisted of 433 (96.7%) patients from the 448 S. mansoni positive patients in the baseline survey (fifteen were lost). Out of the 433, forty were excreting eggs of S. mansoni in their stool. They were treated and left out of the study. Therefore people who remained eligible for the study in this group were 393. Similarly, 468 (94.5%) among the 495 negative patients at the baseline were reviewed (see flow chart below). Ninety two of these 468 previously negative patients were subsequently (6 months later) also found with eggs of S.mansoni in their stool. They were also treated with 40mg/kg body weight and left out of the study. Therefore people who remained eligible for the study in this group were 376. Of the 393 S. mansoni positive reviewed after six months, 83 people were lost to follow up only 310 patients came for follow-up and 80 patients were stool positive for S. mansoni while patients 230 were still stool negative for S. mansoni. Of the 376 S. mansoni negative after six months (not treated), 61 people were lost to follow up only 315 patients came for follow-up, and 102 Patients had become infected with S. mansoni while 213 patients remained stool negative for S. Mansoni throughout the 18 months' follow up. The participants were registered in the three studies using their study code numbers, names, sex, age, home locations and the names of head of the families.

Figure 1: Flow chart showing the follow up of patients from first recruitment up to 18 months
Study area

This study was conducted in Kigungu fishing village, situated along Lake Victoria in Entebbe peninsula. This village is located to the extreme end of the peninsula, at latitude 35° to 38° East and 03° to 07° North. It is about 15 kilometers from Entebbe Municipality and about half a kilometer from the Entebbe international airport. This fishing village was selected because it had base line data on prevalence and intensities of S. mansoni and other soil-transmitted helminths (16). The population of Kigungu is estimated to be 6,000 people with nearly an equal sex 1:1 ratio. Their main occupation is fishing. Besides fishing, they do a little subsistence farming mostly for food crops. Their water exposure is high and hence is the source of infection and reinfection.

Procedure of the study

Informed consent was obtained from all the participants. Residents who had consented to participate in the study and children between 5 and 18 years old who had been granted permission to participate in the study by their parents/guardians and have not taken antischistosomal treatment six months prior to the base line study were registered. This explains the 55 people who were excluded from the base line registration. Those giving their consent and were literate, were asked to sign an official form showing acceptance. Meanwhile the illiterate patients used thumb prints on official form showing acceptance. All participants who consented to the proposed research work. Medical doctors, and technologists carried out laboratory investigations including antischistosomal therapy were not conditioned to the patient’s participation in the study site with praziquantel (Medochemie Ltd.Limassol’Cyprus Europe) at 40 mg per kg body weight. Illnesses, other than schistosomiasis, detected during examination were appropriately treated or referred to other health facilities. Patient’s privacy was duly respected.

Residents for the study reported to the clinic between 9 a.m and 2 p.m of our publicised workdays. They were interviewed and examined by a physician and a nurse during initial screening. The physical examination included special attention to status of the abdominal organs commonly affected by S. mansoni worm. Anaemia and fever were also recorded. Patients with body temperature greater than 37.5°C had blood smear test for malaria parasites done. On each working day, thirty consecutive patients in the order of their arrival at the clinic were registered into the study.

Determination of intensity of infestation by intestinal worms

A stool container labelled with individual code number, name, age and sex was given to each patient to return with about 5-10 gram of stool specimen. Eggs in the stool were quantified using modified Kato-Katz method (17). Essentially, each stool specimen was initially strained through a stainless steel sieve of 250 µm mesh size to remove artefacts. The stained sample was filled in a template, a device measuring 41.7 mg of stool. Each of three separate aliquots of 41.7 mg stool was sampled on a different glass microscope slide and processed for examination as follow. About 10 µl of compound stain consisting of eosin 5% eosin, and 7.5% nigrosin in 10% formalin was added to stool smear on each slide. The stain was stirred in the stool smear on the slide using tooth pick. A wettable cellophane cover slip (32 x 41 mm) pre-soaked in 50% glycerine was carefully placed on the stained stool smear and gently pressed down. The excess stain from the smear on slide was blotted out with absorbent paper before the prepared slide was read using objective x 10 (17, 18). The arithmetic mean of the eggs counted in three slides was recorded as the count in 41.7 milligram of stool. To convert the mean egg count into egg per gram of faeces a factor of 24 was multiplied by the mean of the eggs counted [i.e. number of eggs (n) x 1000 mg/ 41.7 mg = 24 x n = eggs per gram faeces]. Intensity of infection was classified as follows: low = 1-100 eggs per gram, medium = 101-400 and high = ≥ 401 eggs per gram of stool (6).

Data management and statistical analysis

Data were double entered using Microsoft Excel and crosschecked by different researchers. The arithmetic means egg counts of the individuals were categorised according to infection intensities as follows 0-100; 101-200; 201-300; 301-400; and ≥ 401. Similarly age group was also categorised into five groups 5-10; 11-20; 21-30; 31-40 and ≥ 41. The percent of cured proportion was calculated from individuals who had no S. mansoni egg in their stool after treatment divide by the total number of individuals who had S. mansoni egg in their stool before treatment multiply by 100%. The percentage of egg count reduction was calculated from the arithmetic mean egg count after and before treatment. Pearson’s Chi-X² was used to find out associations between a pair of categorical data at 95% level of confidence while Pearson’s and Spearman’s rank correlations were used for comparison of continuous data.

RESULTS

In this study, we recruited nine hundred and forty five residents, adults and children of both sexes. They
were all from Kigungu village of Entebbe in Uganda. Four hundred and forty eight (47.5%) were S. mansoni positive at the baseline survey and they were treated with a single dose of praziquantel at 40 mg/kg body weight. Four hundred and ninety five (52.5%) were negative for S. mansoni. Six months later, the study participants were reviewed for treatment success in clearing the infection. Nine hundred and one (95.5%) of the people who participated in the baseline survey came back for the review but 4.5% (mostly school children relocated themselves elsewhere). A very small proportion (2.4%) of the 448 patients who were S. mansoni positive at the baseline survey continued to excrete eggs of S. mansoni in their faeces. Similarly 2.5% of the 468 negative participants at the baseline survey were infected. The S. mansoni positive participants were again treated with 40 mg/kg body weight and were left out of the study. After follow up of participants from the seventh to the eighteenth months, 625 (310 from positive +315 from negative) of them came back for the third evaluation while additional 276 participants were lost to follow up. Table 1, shows the distribution of intensity of infection among 80 male and female participants who were found infected in the baseline study, treated six months later and still got re-infected and thus found to be stool positive at end of 18 months of follow up. At the same time, the force of clearance by PQZ was 230 (74.2%) [i.e. 230 from 310] this was the total number of patients who were negative for S. mansoni after 18 months. Table 2, Compares difference between those who remained negative after treatment with those who were infected in the baseline study the force of clearance of PZQ showed significant difference (P= 0.00). Similarly the force of clearing the infection of PZQ after 18 was also significant (P=0.00). While the re-infection rate was significant (P=0.0001) after six months there was no significant difference in the re-infection rate after 18 months (P= 0.766).

---

**TABLE 1: INTENSITY OF S. MANSONI AMONGST 80 PATIENTS AT RECRUITMENT AND 18 MONTHS LATER ACROSS AGE GROUP IN YEARS.**

<table>
<thead>
<tr>
<th>S. MANSONI AT RECRUITMENT, N=80</th>
<th>REINFECTION WITH S. MANSONI 18 MONTHS LATER, N=80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td>N</td>
</tr>
<tr>
<td>-----------------</td>
<td>---</td>
</tr>
<tr>
<td>5-10</td>
<td>14</td>
</tr>
<tr>
<td>11-20</td>
<td>45</td>
</tr>
<tr>
<td>21-30</td>
<td>12</td>
</tr>
<tr>
<td>31-40</td>
<td>7</td>
</tr>
<tr>
<td>≥41</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>80</td>
</tr>
</tbody>
</table>

Legend: % = Percentage of infections in the age group. †: Total number of eggs excreted by each age group before and 18 months after the third treatment. The figures in parenthesis represent arithmetic mean of the egg count. N: the total number individuals infected with S. mansoni before and after the third treatment.

**TABLE 2: CURE RATES AND REINFECTION RATES**

<table>
<thead>
<tr>
<th>Cure rates after 6 months</th>
<th>P -value</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>393/433</td>
<td>92/468</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cure rates after 18 months</th>
<th>P -value</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>210/310</td>
<td>102/376</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Re infection rate after 6 months</th>
<th>P -value</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40/432</td>
<td>92/469</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Re infection rate after 18 months</th>
<th>P -value</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80/310</td>
<td>102/376</td>
<td>0.766</td>
</tr>
</tbody>
</table>

Legend: cure rate after 6 months and 18 months against force of reinfection in six months and 18 months after treatment
DISCUSSION

Several studies of infection and reinfection with *Schistosoma mansoni* after treatments with PZQ of residents living in endemic areas have shown that after treatment, prevalence and intensity of parasite infestation due to re-infection are lower than before treatment. In other words, the re-infection prevalence and intensity have been shown never to equal before treatment level (5, 7). In this cohort study to establish regiments for PZQ treatment against *S. mansoni* among the residents of Kigungu, showed that there was reduction in the percentage of infection from 47.4% (448 / 945) to 25.8% (80 / 310) and eggs excretion was reduced by 92.7%. This was 18 months after the initial treatment with PZQ 40mg/kg/wt. We deliberately set a period of 18 months in total with the first six months for the assessment of the success of baseline treatment. We subsequently followed the cohorts twelve months later to allow the study residents exposures to infection. This was to find out the level of resistance and susceptibility developed after treatment. Follow up studies to establish the force of reinfection after treatment with PZQ after short interval is well known (5, 7, 8). Schistosomiasis control Programmes notably Uganda and in other Tropical countries are based on the single dose administration of PZQ to large communities. Repeated treatment to re-infected individuals is recommended depending on the degree of transmission in the areas (8). In spite of the low cost of PZQ most counties in Africa are unable to procure adequate PZQ for mass chemotherapy. In Uganda, the mean cost of treatment per dose per person is $0.3 (2, 19). Study of this kind helps to establish treatment regiments for communities in schistosomiasis endemic areas in the tropical countries with meagre budget for helminth control programmes. Mutapi in their study of changes in specific anti-egg antibody levels with PZQ followed their patients after 9 months (20). In addition, Correa-Oliveira in their natural versus drug induced resistance in *S. mansoni* infections study followed their patients for five years at an interval of one year (7). Repeated treatment with PZQ within short intervals was also found to have no effect on the re-infection period in children in their first decade of life (7). Nevertheless, the cure proportion of PZQ is greater if the treatment is repeated within a short period but the cost of the treatment remains relatively high (6, 10). In this study, in spite of a long period of 18 months after treatment, we were still able to detect a cure proportion of 67.7% (210 out of 310). This observed cure proportion, compare well with others of 60-90% frequently accepted in endemic areas in the tropic using the same PZQ regimen (12, 21). On the other hand lower cure proportion of 18-39% was observed in very intense focus of *S. mansoni* transmission in northern Senegal (4, 22). A set up of this kind may need shorter intervals for re-intervention (7). Pre treatment infections categorised according to the levels of intensity showed that 151 patients in Kigungu were in low intensity levels (1-100 epg), 99 of them were in the middle levels of intensity (101-400epg) and only 60 of them excreted high egg count greater than 401 epg. The observation that 213 patients remained uninfected in all studies points out to an interesting situation in which some of the residents live all their lives in *S. mansoni* endemic areas but do not get the infection. This group of patients commonly known as endemic normal (putative resistance) was always stool negative for *S. mansoni* eggs (23). The putative resistant people of Kigungu have been found to be stool negative for *S. mansoni* before and after treatment (23, 24). Concurrent infection with other intestinal helminths has been shown to affect cure of Praziquantel against schistosomiasis (25). We have observed mixed infection of *S. mansoni* and other intestinal parasites like hookworm (species identification was not done), *Ascaris lumbricoides*, and *Trichuris trichiura*. Our stool analysis was based on a single stool specimen from each patient. In most community-based studies, cure proportion have been estimated based on only one or two slides Kato/Katz reading usually derived from a single stool sample (26). The multiple stool samples procedure is particularly relevant when the overall geometric mean egg count is likely to be low, because it increases the chances of estimating true cure proportion (21).

An unsuccessful effort to eradicate schistosomiasis in the Tropical countries using integrated control methods by World Health Organisation (WHO), lead to the development of morbidity control strategy using antiparasite drugs. However the regiments for treatment for re-infections in different endemic settings remain unclear. In this context we followed our cohorts for 18 months intervening chemotherapeutically using PZQ at base line, after six and 18 months. Reduction in the percentage of infection from 47.4% to 25.8% and reduction of eggs excretion by 69.3% after 18 months raises hope about the long term benefit of praziquantel after treatment in Uganda for large-scale treatment of schistosomiasis.

ACKNOWLEDGEMENTS

This consortium study received financial assistant from the European Union grant SCHISTO-M-VAC for which we are indebted. We are grateful to Miss Nalumansi Zaria and Ester Mirembe for handling our patients professionally. We thank all laboratory personnel from Makerere Medical School for their assistance. The invaluable work of the Local councillors Lukwago David and the late Sempala Edward in mobilizing the residents of Kigungu fishing village to participate in the study is highly appreciated. Similarly, we gratefully acknowledge assistance of the Headmaster (Kigungu Primary School) Mr.Sentongo Mustafa and his staff, school pupils and others who registered voluntarily in this study.
REFERENCES:

COMPARISON OF DERMATOPHYTES AND OTHER AGENTS OF HUMAN DERMATITIS BETWEEN MALES AND FEMALES IN JOS, PLATEAU STATE, NIGERIA

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ABSTRACT

Dermatophytes are a group of three genera of fungi namely Microsporum spp, Trichophyton spp and Epidermophyton spp that commonly cause infections of the skin, hair and nails due to their ability to utilize keratin in both man and animals. Dermatophytes and other agents of human dermatitis are believed to have gender predisposition because of the anatomical and physiological nature of these genders. A study was undertaken to compare the distribution of dermatophytes and other agents of human dermatitis in patients who visited the Dermatophilosis Research Laboratory, National Veterinary Research Institute, Vom, Plateau State of Nigeria. A total of 1551 patients were involved in this study from 2003 to 2007; 823 of whom were males and 728 females. Samples collected were skin scrapings, nails, hair and pus exudates. They were processed according to standard procedures. Nine hundred and thirty two (60%) were positive for dermatophytes and other agents. Microsporum (138 (12.4%)), Aspergillus flavus (128 (11.5%)), and Trichophyton mentagrophytes (112 (10.1%)), Mucor sp (105 (9.5%)) were the most commonly isolated fungi. Aspergillus flavus occurred more in males (74 (6.7%)) while Sporothrix schenckii was more in females (71 (6.4%)). More isolation was made from the head in males (185 (19.8%)) while in females more isolation was made from their limbs (150 (16.1%)). Males generally were more affected with skin infections than females.

INTRODUCTION

Dermatophytes are a group of three genera of fungi namely Microsporum spp, Trichophyton spp and Epidermophyton spp. They commonly cause infections of the skin, hair and nails due to their ability to utilize keratin in both man and animals. They colonize the keratin tissues which results in inflammation caused by host response to metabolic by-products. Occasionally they invade the subcutaneous tissues, resulting in kerion development (1). Dermatophytes are transmitted by either direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in combs, hair brushes, clothing, furniture, public seats, caps, bed linens, towels, and hotel rugs. Approximately 20% of human infections in urban areas are of animal origin while about 80% of human infections in rural areas are of animal origin (2). The dermatophytes are classified as being anthropophilic, zoophilic or geophilic according to their normal habitat. Anthropophilic dermatophytes are those restricted to human hosts and produce a mild, chronic inflammation, zoophilic organisms are found primarily in animals and cause marked inflammatory reactions in humans who have contact with infected cats, dogs, cattle, horses, birds, or other animals. This is followed by a rapid termination of the infection. The geophilic species are usually recovered from the soil but occasionally infect humans and animals. They cause a marked inflammatory reaction, which limits the spread of the infection and may lead to a spontaneous cure but may also leave scars (3).

Dermatophytes and other agents of human dermatitis are believed to have gender predisposition because of the anatomical and physiological nature of these gender. There has also been several research works and resulting publications on the incidences of skin disease as it relates to geographical locations (4, 5 and 6), to the old and sick (7), age groups (8), and occupational groups (9). Most of the studies that have been carried out here in Nigeria have concentrated on children with skin infection (10 and 11). Although Ta’ama et al., (12) looked at the general distribution of dermatophytes in skin lesions among males and females, there has not been much investigation in the aspect of comparing the skin infections that occur in males and females, the organism most prevalent in this infections or the area of the male and female skin most prone to this infections. Knowledge of these occurrences will greatly improve and prevent the high incidence of skin infections in males and females. It is based on these that this study is focused on the comparison of dermatophytes and other agents of human dermatitis between males and females in Jos, Plateau State of Nigeria.
MATERIALS AND METHODS
A total number of 1551 patients visited the Dermatophilosis Research Laboratory, National Veterinary Research Institute, Vom in Jos, Plateau State, Nigeria between 2003 to 2007 with various complaints of skin infections. This was made up of 823 males and 728 females. The consent of the patients was obtained to use their samples for study.

Sample collection and processing
Samples from patients were collected from infected sites of the individuals and this was done by soaking cotton wool in 70% alcohol and swabbing the infected site to disinfect it. Skin scales, nails or hair was scraped using a sterile scalpel blade into clean paper. The pus exudates were collected into a sterile universal bottle. All specimens were labeled properly (name, age, and sex). The collected samples, (the hair and nails were first macerated) were processed by performing an initial wet mount preparation in 20% KOH (Potassium Hydroxide) for direct microscopy as described by (13). Afterwards the samples were seeded into Sabouraud dextrose agar containing chloramphenicol at 16ug/ml using a straight inoculating wire and incubated at room temperature for three to four weeks. The pus was streaked aseptically in blood agar and incubated at 37°C for 24 hours. Gram stain was performed on the resultant culture and viewed using x100 objective. Subsequent bacteria identification was performed to identify the culture. The fungi cultures were identified by their colonial morphology and tease mount method (14).

RESULTS
The results of the study revealed that out of the 1551 patients involved in this study, there were isolations of dermatophytes and other agents of dermatitis from 932 (60%) of the samples collected. Table 1 indicates the array and number of dermatophytes and other agents isolated over the period 2003-2007.

<table>
<thead>
<tr>
<th>Isolated Organisms</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton tonsurans</td>
<td>7</td>
<td>-</td>
<td>7</td>
<td>3</td>
<td>8</td>
<td>25</td>
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<td>Trichophyton rubrum</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>16</td>
</tr>
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<td>4</td>
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<td>34</td>
<td>63</td>
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<td>112</td>
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<td>-</td>
<td>3</td>
<td>21</td>
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<td>28</td>
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<td>-</td>
<td>-</td>
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<td>6</td>
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<td>13</td>
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<tr>
<td>Trichophyton sp</td>
<td>12</td>
<td>5</td>
<td>2</td>
<td>6</td>
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<td>30</td>
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<tr>
<td>Aspergillus fumigates</td>
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<td>14</td>
<td>26</td>
<td>28</td>
<td>15</td>
<td>88</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>31</td>
<td>32</td>
<td>30</td>
<td>2</td>
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<td>128</td>
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<tr>
<td>Aspergillus niger</td>
<td>-</td>
<td>9</td>
<td>11</td>
<td>25</td>
<td>33</td>
<td>80</td>
</tr>
<tr>
<td>Aspergillus sp</td>
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<td>1</td>
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<td>1</td>
<td>5</td>
<td>12</td>
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<tr>
<td>Bipolaris sp</td>
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<td>11</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>37</td>
</tr>
<tr>
<td>Penicillum sp</td>
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<td>17</td>
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<td>1</td>
<td>3</td>
<td>42</td>
</tr>
<tr>
<td>Mucor sp</td>
<td>18</td>
<td>30</td>
<td>24</td>
<td>8</td>
<td>25</td>
<td>105</td>
</tr>
<tr>
<td>Rhizopus sp</td>
<td>11</td>
<td>11</td>
<td>19</td>
<td>4</td>
<td>13</td>
<td>57</td>
</tr>
<tr>
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<td>46</td>
<td>15</td>
<td>4</td>
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<td>138</td>
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<tr>
<td>Microsporum audouini</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
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<td>18</td>
<td>15</td>
<td>3</td>
<td>15</td>
<td>55</td>
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<td>Geotrichum sp</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
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<tr>
<td>Fusarium sp</td>
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<td>9</td>
<td>2</td>
<td>-</td>
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<td>17</td>
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<td>-</td>
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<td>15</td>
</tr>
<tr>
<td>Clostridium</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>6</td>
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<td>Blastomyces dermatolides</td>
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<td>4</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Curularia sp</td>
<td>7</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>12</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>36</td>
</tr>
<tr>
<td>Phialophora verrucosa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Epidermophyton floccosum</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Coccidioides immittis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

The Prevalence of dermatophytes and other agents of human dermatitis in males and females from 2003 to 2007 are shown in Table 2. It states that Aspergillus flavus had the highest occurrence in males within the period of study while Sporothrix schenckii had the highest in females.

Table 3 shows the total number of males and females that attended the dermatophilosis research laboratory for each of the year and the number of positive isolations as well as the percentage isolations. It revealed that more males than females had complaints of skin infection.
Table 4 shows an array of dermatophytes and other agents of human dermatitis isolated based on anatomical position in males and females. This revealed that males had more infection occurring on their heads while the females, had more infections occurring on their limbs.

### TABLE 2: PREVALENCE OF DERMATOPHYTES AND OTHER AGENTS OF DERMATITIS ISOLATES IN MALES AND FEMALES FROM 2003-2007

<table>
<thead>
<tr>
<th>Isolated Organisms</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton tonsurans</em></td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>57</td>
<td>55</td>
</tr>
<tr>
<td><em>Trichophyton violaeum</em></td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td><em>Trichophyton verrucosum</em></td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td><em>Trichophyton sp</em></td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td><em>Aspergillus fumigates</em></td>
<td>54</td>
<td>34</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>74</td>
<td>49</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td><em>Aspergillus sp</em></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><em>Bipolaris sp</em></td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td><em>Penicillium sp</em></td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td><em>Mucor sp</em></td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td><em>Rhizopus sp</em></td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td><em>Sporothricum schenckii</em></td>
<td>67</td>
<td>71</td>
</tr>
<tr>
<td><em>Microsporum audouini</em></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>36</td>
<td>19</td>
</tr>
<tr>
<td><em>Geotricum sp</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Fusarium sp</em></td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td><em>Scopulariopsis sp</em></td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td><em>Clostridium sp</em></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><em>Blastomyces dermatitides</em></td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td><em>Curcularia sp</em></td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td><em>Cladosporium sp</em></td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td><em>Phialophora vemucosa</em></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><em>Epidermophyton floccosum</em></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><em>Coccidiodes immitis</em></td>
<td>-</td>
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</tr>
</tbody>
</table>

### TABLE 3: SUMMARY OF INFECTION IN MALES AND FEMALES FROM 2003 TO 2007

<table>
<thead>
<tr>
<th>Year</th>
<th>Number on roll</th>
<th>Number of +ve Isolation</th>
<th>% of +ve Isolation</th>
<th>Number on roll</th>
<th>Number of +ve Isolation</th>
<th>% of +ve Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>164</td>
<td>109</td>
<td>20.9%</td>
<td>114</td>
<td>70</td>
<td>17.03%</td>
</tr>
<tr>
<td>2004</td>
<td>206</td>
<td>111</td>
<td>21.3%</td>
<td>150</td>
<td>70</td>
<td>17.03%</td>
</tr>
<tr>
<td>2005</td>
<td>164</td>
<td>103</td>
<td>19.8%</td>
<td>195</td>
<td>99</td>
<td>24.09%</td>
</tr>
<tr>
<td>2006</td>
<td>126</td>
<td>77</td>
<td>14.8%</td>
<td>142</td>
<td>88</td>
<td>21.41%</td>
</tr>
<tr>
<td>2007</td>
<td>163</td>
<td>121</td>
<td>23.2%</td>
<td>127</td>
<td>84</td>
<td>20.44%</td>
</tr>
<tr>
<td>Total</td>
<td>823</td>
<td>521</td>
<td>100%</td>
<td>728</td>
<td>411</td>
<td>100%</td>
</tr>
</tbody>
</table>
DISCUSSION
The result obtained from this study has revealed a wide range of dermatophytes and other agents of human dermatitis that occur in both males and females. Two zoophilic species of dermatophytes, *T. mentagrophytes* and *T. verrucosum*, which are known to be fundamentally pathogens but sometimes cause disease in man, were isolated from this study. Anthropophilic species of dermatophytes *T. rubrum*, *T. tonsurans* and *M. audouinii*, which are mainly human pathogens but occasionally infect animals, were also isolated as well as other mould fungi from both sexes. This suggests that prevailing endemic pathogens among different species of living things have the ability to change with time in respect to the existing living and hygiene conditions.

In this study, more males, that is 521 out of 823 which gives a 63.3%, had positive isolation of dermatophytes and other fungi more than the females which had 411 positive isolations out of 728, giving a 56.5% of positive isolation. This could be as a result of the male skin which unlike the female skin has larger pores, a richer blood supply, and more active sebaceous glands. This means that the male skin is more prone to sweating and tends to be on the oilier side and as a result, male skin is, in fact, dirtier than female skin (15). The differing activities performed by most males and females in terms of the kind of jobs they do, period of exposure to the elements of weather and the care and attention given to the skin is also a contributing factor to the result gotten. Field and laboratory studies have linked this increased susceptibility to infection with sex hormones, hormonal and immunological differences or mechanisms of the sexes (16, 17 and 18). Gender-determined differences in susceptibility to virus infections have been reported for encephalomyocarditis virus (19). Females are known to mount more vigorous immune responses, especially humoral responses, and in general show higher resistance to bacterial and viral infections (20 and 21).

Even the prevalence and intensity of infections caused by protozoa, nematodes, trematodes, cestodes, and arthropods is said to be higher in males than females, (22). This then raises the possibility of this phenomenon with fungal infections. Animals are not left out in this fact as studies of rodent malaria have illustrated that mortality rates are higher in males compared with females and may involve endocrine and immunological differences between the sex (22).

Males in this study recorded more infection occurring on the head region which comprises of the scalp, face and neck. The females on the other hand recorded their highest site of infection on the limbs (hands and legs). Some of the reasons for the high occurrence of infection on the heads of males could be due to the shortness of their hair, which facilitates easy reach of the fungal spores to the scalp. The frequent shaving of hair from their head and beard from their face by different people, instruments and at different saloons could make them more susceptible to infections. This routine shaving destroys the hydrolipidic film, leading to loss of natural lubrication and protection. This is in complete contrast to females who do more of washing and maintaining of their hair. Another factor could be the less concern the males have about the appearance of their face compared to the females who are very conscious of their face and hair and as such are able to notice and act promptly therefore minimizing infections from their heads, face and neck.

In the case of the females who recorded more infections on their limbs, the reason could be that the female limbs are exposed to damaging environmental elements daily as a result of the nature of their dressings. This is in contrast to their male counterparts whose limbs are mostly covered in trousers and long-sleeved shirts. This study clearly shows that a higher occurrence of dermatitis is more likely to be seen in males than females for two basic reasons. First is that females are more socially and psychologically conscious of their appearance and so will notice and seek medical advice faster than the males who are likely to not even notice the skin problem on time or when they eventually do, will only seek treatment when there is no other alternative. The second reason is the fragility of the male skin especially on the face as a result of less care of the skin and constant exposure to damaging environmental elements. Although it has been extensively proved that the sex hormones in females whether human or animal make them more resistant to bacterial and viral infections (20 and 21), its application to fungal infections is still to be fully

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**TABLE 4: FREQUENCY OF INFECTION IN THE ANATOMY OF MALES AND FEMALES FROM 2003 TO 2007**

<table>
<thead>
<tr>
<th>Area of the body</th>
<th>General (all over the body)</th>
<th>Limbs (hands &amp; legs)</th>
<th>Head (head, face &amp; neck)</th>
<th>Trunk (shoulder to neck)</th>
<th>Folds (armpit/knee)</th>
<th>Nails (toe &amp; finger)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td><strong>Male</strong></td>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>90</td>
<td>95</td>
<td>176</td>
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<td>185</td>
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</tr>
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<td>185</td>
<td>10</td>
<td>44</td>
<td>5</td>
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<td>5</td>
</tr>
</tbody>
</table>

---

88
studied. This is definitely an area of research that needs further study.

ACKNOWLEDGEMENT

REFERENCES


The authors wish to acknowledge Mr. Bulus Datok, Mrs M. Nanza and Miss S. Yusfu for their technical assistance.
EVALUATING THE EFFICACY OF TOPICAL SILVER NITRATE AND INTRAMUSCULAR ANTIMONIAL DRUGS IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS IN SOKOTO, NIGERIA

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ABSTRACT
Background: Cutaneous leishmaniasis (CL) is a disease of public health importance in Nigeria, with high prevalence in the Northwest and Northeastern part of the country. The side effects of antimonial drugs [stibogluconate (SSG) and meglumine antimoniate] in the treatment of CL have often resulted in poor drug adherence and default by patients and possible drug resistance. The increasing default to follow-up and the significant side effects associated with antimonial therapy necessitated the dire need of alternative therapeutic modalities. Thus, this study aimed at comparing the efficacy of topical silver nitrate with the antimonial drugs in the treatment of CL.

Methods: A total of 95 patients with clinically diagnosed leishmaniasis and parasitologically proven CL participated in the study after their informed-consent had been obtained. The treatment selection was optional to the participants. Sixty (63.2%) patients received alternative therapy of topical silver nitrate as a single dose while 35(36.8%) patients received antimonial therapy for 21 days at 20mg/kg body weight.

Results: On day 30 of treatment, 68 (86.1%) lesions among patients on topical silver nitrate healed completely as compared with only 5 (6.8%) among those on i.m. SSG. There was no improvement in 25 (34.2%) lesions among those on i.m. SSG compared with only 1 (1.2%) lesion among those on topical silver nitrate. Overall, there was a statistically significant difference in the cure rate among patients on silver nitrate as compared with those on i.m. SSG on the 21st and 30th days of treatment.(p<0.05).

Conclusion: Topical silver nitrate therapy is an effective and better drug treatment for CL among this study population.

Keywords: Cutaneous leishmaniasis, efficacy, Silver nitrate, Sodium Stibogluconate, Sokoto

INTRODUCTION
Leishmaniasis is an infection that is caused by an obligate intracellular protozoa of the genus *Leishmania*; first described in 1903 by Leishman and Donovan. The natural transmission of *Leishmania* parasites is carried out by sandflies of the genus *Phlebotomus* or *Lutzomyia* (1). These parasites cause three forms of leishmaniasis according to the localization of the parasites in mammalian tissues, notably visceral, cutaneous, and mucosal leishmaniasis. The outcome of infection depends on the species of *Leishmania*.
parasites and the host’s immune response (2). Visceral leishmaniasis (VL) is the most severe form of leishmaniasis involving internal organs such as the spleen and liver and is fatal if left untreated (3).

Globally, about 350 million people live in areas of active transmission of *Leishmania*, with estimated 14 million people directly affected in Africa, Asia, Europe, and America (4). The global burden of leishmaniasis has remained stable for some years, causing a morbidity and mortality loss of 2.4 million Disability Adjusted Life-Years (DALYs) and approximately 70,000 deaths; a significantly high figure ranked among communicable diseases (5,6). Leishmaniasis and Human Immunodeficiency Virus (HIV) co-infection has surged as a major complication of leishmaniasis and has ignited calls for the recognition of leishmaniasis as an Acquired Immunodeficiency Syndrome (AIDS) defining-illness (7). In Africa, particularly Ethiopia and Sudan, it is estimated that 70% of adults with VL also have HIV infection (8).

Northern Nigeria is one of the West African endemic CL foci, especially in Sokoto, Gusau, Katsina, Maiduguri and Azare9. Between 1924 and 1941, the Nigeria health services recorded 131 cases of CL and 5 cases of VL, but there was no indication that these cases were parasitologically confirmed. In 1942, smears of 14 patients from Kano with cutaneous sores were confirmed parasitologically to be leishmaniasis. However, the first case of CL in Nigeria was officially reported in 1944 (9,10). In spite of the initial public health efforts on CL in this region, there is dearth of follow-up data on CL caused by *Leishmania tropica* and its treatment-induced cure rates.

The current management of leishmaniasis are drug treatment of patients, to alleviate disease and vector control to reduce its transmission. Pentavalent antimonials (namely, sodium stibogluconate (SSG) and meglumine antimoniate) are the mainstay of anti-leishmanial therapy (11-13). However, the antimonials have serious adverse effects (such as pancreatitis, hepatotoxicity, and cardiotoxicity) when given intramuscularly (14,16) and are expensive (17). Moreover, the lengthy course of its administration and associated pain often results in low drug adherence, default and ultimately, drug resistance (18,18).  Bearing in mind several studies that have shown the high efficacy of colloidal silver as an antimicrobial (20-23), the topical silver nitrate appears to be the most feasible alternative. Silver is toxic to microorganisms by poisoning respiratory enzymes and components of the microbial electron transport system as well as impairing some DNA function24-25. Additionally, silver has been shown to interact with structural proteins and preferentially bind with DNA bases to inhibit replication (26,27).

Therefore, in order to achieve effective control of CL, it is pertinent to try other drug-treatment modalities which are painless, tolerable, and of short duration. Hence the focus of this study which compared the efficacy of the topical silver nitrate therapy with the antimonials in the treatment of CL due to *L. tropica* in Sokoto, Northwest Nigeria.
MATERIALS AND METHODS

Study location and Participants. The study was carried out at 1 Brigade Medical Centre which is located in Gingiya military barracks, Sokoto, Northwest Nigeria. Established in 1984, the medical centre is well staffed, with equipped laboratory and provides curative and preventive services to the soldiers, their families and the civil populace. It is also a designated centre by the Sokoto State Ministry of Health and the Nigerian Ministry of Defence-US Department of Defense (NMOD-USDoD) for the treatment of Leishmaniasis and HIV/AID respectively.

Ninety five patients, aged more than 5 years with parasitologically confirmed CL lesions were recruited for the study. The exclusion criteria were pregnancy, breast-feeding mothers, patients who had a major surgery in the previous 3 months, presence of any uncontrolled medical condition; anticipated unavailability for follow-up; and patients allergic to antimonials and silver nitrate.

Ethical issues: The authority of the Medical Centre and State’s ethics committee approved the study protocol and the consent form. New and eligible patients attending the clinic for treatment were briefed about the aim and protocol of the study. Patients were enrolled in the study after an informed consent had been given. All medical services provided during the study were free.

Participant’s Medical History: Prior to the study, a detailed medical and personal history of each participant was taken. These included the duration of symptoms, residence in or travel through a possibly leishmaniasis-endemic area, history of any existing health condition, menstrual and parity history, previous treatments and outcome. A general physical examination was also carried out to assess the location, size of lesions with other characteristics such as infiltration, erythema, scaling and ulceration.

Parasitological confirmation: Parasitological confirmation of CL was by microscopic examination of smear and with RIDASCREEN leishmania antibody test kit (R-Biopharm, Germany). Scrapings from the lesion edge were smeared onto a slide, and the slide was dried, fixed with methanol, stained with Giemsa and examined under the microscope at x100 magnification for presence of Leishmania amastigotes. For each, patient, 2 slides were prepared and all positive slides were rechecked and confirmed by at least 2 laboratory scientists.

For leishmania antibody testing about 3mls of whole blood was collected from each participant into a clean dry plain blood container and allowed to clot. The blood sample was spun with a bench centrifuge at 5000rpm for 5 minutes and the serum separated into another clean plain container and stored frozen prior to testing at -20°C. The samples were tested using Elisa leishmania antibody kit (R-Biopharm, Germany), using the procedure as described in the manual of the kit.

Treatment Procedure

Sixty patients had a single dose topical silver nitrate treatment while 35 had daily intramuscular administration of SSG for 21 days using the World Health Organization recommended treatment for CL (28). For intramuscular administration, 20 mg/kg of SSG was administered daily (up to a maximum daily dose of 850 mg) for 21 days (28). For silver nitrate
treatment, the surface of the each lesion was cleaned with hydrogen peroxide solution and scraped with scalpel blade. The lesion surface was mopped with cotton wool to prevent bleeding; fine granules of silver nitrate were poured into it, covered with water-soaked cotton wool and dressed with plaster for 24 hours. After 24 hours, each lesion was cleaned daily with hydrogen peroxide and covered with Gentamycin-ointment smeared bandage for 21 days. Also, in order to prevent secondary infection, each patient on the topical silver nitrate had 280 mg of intramuscular Gentamycin for 5 days. Treatment efficacy was measured by the percentage of patients cured on 21st and 30th day after commencing treatment. “Cure” is defined as “the complete re-epithelialization of the CL lesion, with no evidence of papules, inflammation, or indurations”.

Data collation and analysis.. The obtained data were analyzed using EPI info computer software package. The results were displayed in tables and Chi square test statistic was used at 95% level of significance.

RESULTS
A total of 95 patients comprising 74 males (77.9%) and 21 females (22.1%) were recruited for the study as shown in Table 1. Thirty five patients (with a total of 73 lesions) received i.m. SSG while 60 (with a total of 79 lesions) received topical silver nitrate treatment. In both groups, each male patient had an average of 1.2 lesions while each female had an average of 2.5 lesions; thus implying that the lesions were more among females than males. The age range of the patients who had intramuscular SSG was 31-42 years (mean = 30.5) while that of patients who had topical silver nitrate was 16-52 years (mean = 32.4).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intramuscular SSG (%)</th>
<th>Topical Silver Nitrate (%)</th>
<th>Total (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>No of Patients</td>
<td>30 (31.6%)</td>
<td>5 (5.3)</td>
<td>44 (46.3%)</td>
</tr>
<tr>
<td>No of Lesions</td>
<td>44(28.9)</td>
<td>29(19.1)</td>
<td>41(27%)</td>
</tr>
<tr>
<td>Mean Age and Range (in years)</td>
<td>30.5 (13 - 42)</td>
<td>32.4 (16 - 52)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 showed that the nodular/nodular-ulcerative lesions were the commonest (constituting 82.1% and 73.4% in i.m. SSG and topical silver nitrate groups respectively). Over two-third lesions were located in the upper limbs in each treatment category (i.m. SSG=65.8%, topical silver nitrate=68.3%). No lesion was seen in the groin, perineum or buttocks in both treatment categories.

Table 3 showed the cure rate in each category after 21st and 30th day of treatment. On day 21, Sixty five (82.3%) lesions among patients on topical silver nitrate healed completely compared
with 4 (5.5%) among those on i.m. SSG. There was no improvement in 30 (41%) lesions of patients on i.m. SSG compared with 4 (5.1) among those on topical silver nitrate. On day 30 of treatment, 68 (86.1%) lesions among patients on topical silver nitrate healed completely as compared with only 5 (6.8%) among those on i.m. SSG. Also, there was no improvement in 25 (34.2%) lesions among those on i.m. SSG compared with only 1 (1.2%) lesion among those on topical silver nitrate. There was a statistically significant difference in the cure rate among patients on silver nitrate as compared with those on i.m. SSG on the 21st and 30th days of treatment. (p<0.05).

Apart from complaints of pain at the site of injections, which was generally greater in the patients who were given i.m SSG, the medications were well-tolerated. For each patient, the results of the post-treatment biochemical and hematological investigations were normal.

### TABLE 2: CHARACTERISTICS OF THE CUTANEOUS LEISHMANIASIS LESIONS OF THE 95 PATIENTS TREATED

<table>
<thead>
<tr>
<th>Characteristics of Lesions</th>
<th>Type of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intramuscular SSG (n = 73 lesions)</td>
</tr>
<tr>
<td>Mean area of lesion and range (in cm²)</td>
<td>1.8(0.6-4.6)</td>
</tr>
<tr>
<td>Lesion type (% of lesions)</td>
<td></td>
</tr>
<tr>
<td>Nodular</td>
<td>32(43.8)</td>
</tr>
<tr>
<td>Nodular-ulcerative</td>
<td>28(38.3)</td>
</tr>
<tr>
<td>Flat-ulcerative</td>
<td>10(13.7)</td>
</tr>
<tr>
<td>Plaque-like</td>
<td>3(4.1)</td>
</tr>
<tr>
<td>Lesion site (% of lesion)</td>
<td></td>
</tr>
<tr>
<td>Shoulder</td>
<td></td>
</tr>
<tr>
<td>Upper arm</td>
<td>8(10.9)</td>
</tr>
<tr>
<td>Elbow</td>
<td>10(13.7)</td>
</tr>
<tr>
<td>Lower arm</td>
<td>3(4.1)</td>
</tr>
<tr>
<td>Hand</td>
<td>18(24.7)</td>
</tr>
<tr>
<td>Thigh</td>
<td>9(12.3)</td>
</tr>
<tr>
<td>Knee</td>
<td>4(5.5)</td>
</tr>
<tr>
<td>Leg</td>
<td>2(2.7)</td>
</tr>
<tr>
<td>Foot</td>
<td>13(17.8)</td>
</tr>
<tr>
<td></td>
<td>6(8.2)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

There have been several attempts in the past to develop a safe and simply administered drug with a broad spectrum of activity against CL. For example, high cost and painful injections are documented drawbacks of treatment by antimonial compounds(14,15). In addition, the antimonial drugs have been documented to have 80-85% cure rate, recurrent clinical signs and relapses (16). Thus, the dire need to develop effective compounds for the treatment of CL that would be economical, ease of administration and
could avoid resistance becomes expeditious. To surmount this challenge, topical/local treatment of CL lesions seems a logical alternative. Although some forms of topical/local therapy have been in long use in endemic areas by traditional practitioners, they had little if any effect (27).

In this study, more than three-quarters of the selected patients were males. The few female participants could be attributed to the exclusion criteria which included pregnant women and breastfeeding mothers. The patients were given the option of choosing a treatment regimen after the informed consent had been obtained and this accounted for the selection of silver nitrate treatment by 60 (63.2%) of the 95 patients. For each treatment regimen, it was observed that the male patients had an average of 1-2 lesions while females had an average of 2-5 lesions; thus implying that the lesions were more among females than males. The reason(s) for this variation cannot be explained since both gender were equally exposed to the vector. Further research to determine this variation is desirable. Also, in both treatment regimen, it was observed that over two-thirds lesions were located in the upper limbs (i.m. SSG=65.8%, topical silver nitrate=68.3%) and none was seen in the groin, perineum or buttocks. This finding implies that the exposed parts of the body were more accessible to the vector. It is therefore necessary to emphasize the use of protective clothes by the communities; especially while in the farm (or grazing animals); as an important preventive measure against the vector bites.

<table>
<thead>
<tr>
<th>Duration (in days)</th>
<th>Cure Rate</th>
<th>IM SSG (%) (n=73)</th>
<th>Topical Silver Nitrate (%) (n=79)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 21 of treatment</strong></td>
<td>Fully healed lesions</td>
<td>4 (5.5)</td>
<td>65 (82.3)</td>
<td>0.00000</td>
</tr>
<tr>
<td></td>
<td>Improved lesions</td>
<td>39 (53.4)</td>
<td>10 (12.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No change in lesion</td>
<td>25 (34.2)</td>
<td>4 (5.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsened lesion</td>
<td>5 (6.8)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Day 30 of treatment</strong></td>
<td>Fully healed lesions</td>
<td>5 (6.8)</td>
<td>68 (86.1)</td>
<td>0.00000</td>
</tr>
<tr>
<td></td>
<td>Improved lesions</td>
<td>43 (58.9)</td>
<td>10 (12.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No change in lesion</td>
<td>21 (28.8)</td>
<td>1 (1.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsened lesion</td>
<td>4 (5.4)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

More than four-fifth (82.3%) of the lesions among patients on topical silver nitrate and only 5.5% lesions those on i.m. SSG healed completely on the 21st day of commencing treatment. By the
30th day, 86.1% of the lesions among those on silver nitrate had healed completely as compared with only 6.8% among those on i.m. SSG. There was a statistically significant difference in the cure rate between patients on silver nitrate and those on i.m. SSG on the 21st and 30th days of treatment. (p<0.05).

**Conclusion:** In comparing the efficacy of topical silver nitrate and i.m. SSG in the treatment of CL, this study has shown that a single dose treatment with topical silver nitrate plus topical gentamycin (for secondary infections) was more effective. The study also highlighted the need to educate the community on the use of protective clothing to reduce the bite by the vector. The reasons for the gender variation in the number of CL lesions among this study needs to be further researched.

**ACKNOWLEDGMENT**
We are grateful to the management and staff of 1 Brigade Medical Centre, Gingiya Barracks for their facility and cooperation during the period of the study.

**REFERENCES**


REPRODUCTIVE HEALTH ISSUES AND INCIDENCE OF SOME REPRODUCTIVE TRACT INFECTIONS AMONG MUSLIM WOMEN IN PURDAH IN JOS-NIGERIA

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Correspondence: Dr. K. B. Tanyigna

ABSTRACT
Candidiasis and Trichomoniasis are the commonest Reproductive Tract Infections (RTIs) amongst women in purdah in the capital city of Jos- Nigeria. Majority of these women (>68%) have primary education as the highest educational qualification and majority of them (>92%) are full time housewives. The study has also revealed that there is a high level (57%) delay in seeking for reproductive health needs, which were only remedied due to persistence of symptoms. This gives us the inference as to the myriad of sequelae that might have resulted in cases of asymptomatic RTIs. The most reproductive health needs of women in purdah are inadequate facilities in clinics/hospitals. This factor as well as other factors such as poor education, unilateral decision making by some men in purdah in matters of reproductive needs, misunderstanding and misinterpretation of the Qu’ran have immensely contributed to the increasing cases of RTIs among these women. These problems can be reduced to the minimum by educating both the men and the women in purdah with the best medium being the radio/television programs.

KEY WORDS: Purdah, Reproductive Health, Infection, Women.

INTRODUCTION
Purdah is the practice of seclusion and veiling among women. This practice is seen mostly among the Muslim. This custom is however shared by the Hindus and Muslims in what is usually considered the Purdah zone. This zone comprises Bangladesh, Pakistan and India. The purposes for the practice among these two religions are different. For the Muslims the purpose is to safeguard their women from view outside the family and to keep them in their own separate feminine world for the purpose of maintaining fidelity (1, 2). In stating the reasons for the need of purdah, the Qu’ran declares that “the observance (of hijab is so that the (pure and pious women) may be recognized and not be molested” (3).

Increasing number of Muslim women in purdah in Jos, the capital of Plateau State in Nigeria have been noticed visiting hospitals and clinics with chronic cases of Reproductive Tract Infections (RTIs) or Sexually Transmissible Diseases (STDs) are the reasons for the chronic infections related to restrictions of movements placed on these women? Or are they related to the decision making process? Answers to these questions and many others are attempted in the cause of this study.

Motivation is an entity that compels one to action of a particular type, i a desire that precedes an act and determines it (4). From the instinctive point of view, it means that motivation consists of energy generation and direction guiding processes which together produce all forms of human behavior. Health — care seeking motivation is therefore the desire to seek for health. This involves the drive as to why, and the mechanism as to how one goes about seeking for health care (5). The outcome of these complex systems of motives or motivations is human behavior (6). If purdah women now go to seek for health care in spite of the restrictions on movement hence these increasing visitations to hosptals and clinics, several motives may be responsible for these compelling actions. The woman may want to be cured of the disease, or may be aware of the risk, or may even want to be adorned by her husband. There may be several other reasons which this study is aimed at highlighting.

Reproductive Tract Infections can be sexually transmitted or non-sexually transmitted but have grave sequelae especially if untreated. These sequelae include: Pelvic Inflammatory Disease, spontaneous abortion and still birth, low birth weight, congenital infection, ectopic pregnancy, infant blindness and mental retardation (7, 8, 9, 10). Among the non pregnant women the sequelae include: cancers (11); infertility in about 50 – 80% (12) and genital infections is said to result to 90% maternal death in developing countries (13).

The incidences of some of these RTIs have also been studied in comparison to women not in purdah practice. This is because RTIs do not only bring about medical problems but they also constitute social and economic problems in Nigeria not only among urban dwellers but in the rural areas as well (14). Gonorrhea, Syphilis and now HIV/AIDS are the most widely known RTIs/STDs but there are more than twenty others. On the average, an estimated 685,000 people are infected daily with these diseases and every year there are about 250 million new cases (15).
MATERIALS AND METHODS

Selection of subjects and Specimen Collection
After an official permission had been sought and obtained from Shifa hospital in Jos, 85 women in purdah visiting the hospital with suspected cases of RTIs were examined and high vaginal swabs (HVS) were collected by the Medical Officer from the upper third wall of the vagina with a sterile swab stick. The swab was rotated gently against the vagina wall. This was after a sterile moistened speculum had been inserted into the vagina to expose the cervix. Specimen collection was limited to HVS so as to limit the scope of the research.

Inoculation of samples
Specimen was applied to a small area of the prepared (MacConkey, Chocolate and Sabouraud) agar plates to make wells. A flame sterilized wire loop was then used to spread the inoculum to ensure single colonies and incubated for 24 hours at 37°C.

Preparation of wet smears
A sample of the exudates on the swab stick was transferred on a clean glass slide and a drop of physiological saline was added and mixed properly to make a thin preparation. This was covered with a cover slip and then examined under the microscope using x10 and x40 objectives of the microscope. This method was aimed at identifying parasites and protozoan RTIs according to Cheesbrough (16).

Gram Staining of Smears
After labeling the clean slide with patients name and date, the swab containing the specimen was then rolled on the clean slide and spread evenly covering an area of about 15mm in diameter to make a smear. The smear was left in a safe place to air-dry and then passed through a burning flame three times and allowed to cool. The slide was then laid on a staining rack and covered with crystal violet stain for 60 minutes and rapidly washed off with clean water. The water was tipped from the slide and the smear covered with Lugol’s iodine for 60 seconds and washed off. Afterwards the smear was decolourised rapidly with acetone and washed off immediately. The smear was then covered with neutral red for 2 minutes and washed off with water. The slide was finally placed on a staining rack to air-dry before being viewed using x40 and x100 objectives of the microscope.

Vaginal fluid pH and whiff (Amine) Tests
The pH paper was placed on the surface of the speculum after it had been removed from the vagina and the pH read, while the vaginal malodor was used to diagnose *Gardnerella vaginosis*. The method included the release of “fishy” amines from the vaginal fluid after the addition of 10% potassium hydroxide (KOH). Swabs containing vaginal fluid were rolled on clean slides and KOH were added.

Structured in-depth Interview
A structured questionnaire (appendix 1) was administered on each of the women involved in the study to collect various data on Health-seeking motivations, Reproductive health needs, Decision making process and the knowledge of RTIs among women in purdah.

RESULTS
The incidence rates of RTIs among the 85 respondents were as follows: 
*Candida* spp had 75 (88.2%); *Trichomonas vaginalis* with 3 (3.7%) and *Gardnerella vaginalis* with 2 (2.4%). There were no infection in 5 (5.9%) of the cases (Table I).

Similarly the age distribution showed that age group 21- 30 attended the hospital most with suspected cases of RTIs. This age group had 68 (80%) cases (Table 1).

---

### TABLE 1: RTIs AMONG DIFFERENT AGE GROUPS OF WOMEN IN PURDAH VISITING THE HOSPITAL

<table>
<thead>
<tr>
<th>Age group/RTI</th>
<th>11-20(%)</th>
<th>21-30(%)</th>
<th>31-40(%)</th>
<th>Total(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida</em> spp</td>
<td>8 (9.4)</td>
<td>58(68.2)</td>
<td>9(10.6)</td>
<td>75(88.2)</td>
</tr>
<tr>
<td><em>Trichomonas vaginalis</em></td>
<td>0 (0)</td>
<td>3 (3.5)</td>
<td>0 (0)</td>
<td>3 (3.5)</td>
</tr>
<tr>
<td><em>Gardnerella vaginalis</em></td>
<td>0(0)</td>
<td>2 (2.4)</td>
<td>0 (0)</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>No infection</td>
<td>0(0)</td>
<td>5 (5.9)</td>
<td>0 (0)</td>
<td>5 (5.9)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (9.4)</td>
<td>68 (80)</td>
<td>9 (10.6)</td>
<td>85 (100)</td>
</tr>
</tbody>
</table>
Table 2: Marital status of women in purdah visiting the hospital with symptoms of RTIs

<table>
<thead>
<tr>
<th>RTI</th>
<th>Married (%)</th>
<th>Divorced (%)</th>
<th>Single (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida spp</td>
<td>71 (83.5)</td>
<td>2 (2.35)</td>
<td>2 (2.4)</td>
<td>75 (88.2)</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>3 (3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (3.5)</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>2 (2.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>No infection</td>
<td>4 (4.7)</td>
<td>1 (1.27)</td>
<td>0 (0)</td>
<td>5 (5.9)</td>
</tr>
<tr>
<td>Total</td>
<td>80 (94.1)</td>
<td>3 (3.5)</td>
<td>2 (2.4)</td>
<td>85 (100)</td>
</tr>
</tbody>
</table>

Table 3: Educational status of women in purdah visiting the hospital with symptoms of RTIs

<table>
<thead>
<tr>
<th>Highest Education</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>58 (8.2)</td>
</tr>
<tr>
<td>Secondary</td>
<td>16 (18.8)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>Qur'anic</td>
<td>8 (9.4)</td>
</tr>
<tr>
<td>No formal education</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Total</td>
<td>85 (100)</td>
</tr>
</tbody>
</table>

Table 2 shows that 80 (94.1%) of the respondents were married while 3 (3.5%) were divorced and 2 (2.4%) were single. Most of these women (68.2%) had their educational status at the primary level (Table 3). So also were most of the women (92.9%) full time housewives (Table 4).

At the earliest notice of symptoms of RTIs, a total of 49 (57.6%) of these cases delayed between the periods of less than 1 month and more than 1 year before presenting to the hospital, while 36 (42.4%) presented their cases at the earliest noticed of the symptoms although 89.8% of those that delayed were not aware of the risk involved in the delay. Others who were aware of the risk but delayed going to hospitals were on the basis that they went to traditional healers or administered self-treatment. On the other hand, those that did not delay in having prompt treatment after noticing the symptoms because they were aware of the risks were 13 (36.1%).

There are several reproductive health needs of these women but the most (i.e. 51.76%) being the lack of adequate facilities for these women followed by the issue of abortion and miscarriage of pregnancies with 18.82%. The best ways in which decision making process can be altered in favour of these women in purdah appears to be by educating both women in purdah and their husbands. This method of education is suggested by 89.4% of these women. Similarly 45.1% of these women have suggested that they can best be educated on matters that have to do with RTIs through Radio/Television and Health personnel respectively.

DISCUSSION

The Reproductive Tract Infections (RTIs) with the highest rates in the present study are Candidiasis and Trichomoniasis which is in agreement with the work of Ogunbanjo (14) who worked in the Northern and Southern parts of Nigeria. However, the work of Wasserheit (17) showed Gonorrhea to be the commonest RTI in African countries. The difference in the findings may not be unrelated to the fact that diagnostic procedures in the present study were biased against gonorrhoea due to the limitation of materials. The high rate of Candidiasis in purdah and indeed our community could be understood if factors which favour the growth of Candidiasis such as use of scented soaps, poor hygiene, and vaginal disinfectant are seen to also have high rates in our community.

The high incidence rates of trichomoniasis may also be understood against the background that most of it is asymptomatic in women (11) although it is clinically indicated by severe itching and painful sexual intercourse.

Majority of purdah women as reflected in the present study showed that their highest level of education is the primary (68.20%) as well as the fact that majority were full time housewives with low income (92.90%). This does not come as a surprise because education in the core north has been focused mainly on men due to religion and social community sanctions (18). Again since girls at the age of 12 and 13 could be given out to marriage coupled with the fact that purdah reduces female mobility.

The low level of education in purdah can also be related to the high percentage (57%) of purdah women who delay seeking for health care in matters of RTIs as well as their poor sanity. Sufficient education could have made for the sequelae of the infections and adequate sanity precautions not only to be known but also taken rather than going to traditional healers and use of hot water to cure RTIs. This is also why the motivating factors for eventually seeking for reproductive health were the persistence of symptoms.
The low socio-economic status of the women in *purdah* as manifested in this study is also a suggestion as to why these women do not plan for the children to bear and are not knowledgeable about RTIs. This has equally had effect on the spread and the chronic state of RTIs because they share the toilets and bathrooms.

As a result of the fact that Nigerians from the northern part are good listeners of radio/television programmes, this means of communication is hereby suggested as a way through which women in *purdah* can be educated on reproductive health matters. This has also been reflected by the suggestions of the women in the structured interview questionnaires.

The greatest reproductive health need of women in *purdah* as revealed in this study has to do with inadequate facilities in clinics/hospitals. Adequate facilities would make these women feel comfortable. This is because when women felt that they have a secured environment, they are likely to reveal much more information about themselves. This is agreed by Oomman (19) who stated that other equally important issues are usually missing in reproductive health literature. Oomman (19) further stated that sexuality is a reflection of a complex phenomenon with a comprehensive list of elements.

**TABLE 4: OCCUPATIONAL STATUS OF WOMEN IN PURDAH VISITING THE HOSPITAL WITH SYMPTOMS OF RTIs**

<table>
<thead>
<tr>
<th>RTI</th>
<th>Full time</th>
<th>Business</th>
<th>Civil</th>
<th>Student</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida spp</td>
<td>71(82.3)</td>
<td>2(2.35)</td>
<td>1(1.2)</td>
<td>2(2.4)</td>
<td>75(88.2)</td>
</tr>
<tr>
<td>T. Vaginalis</td>
<td>3(3.5)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>3(3.5)</td>
</tr>
<tr>
<td>G. Vaginalis</td>
<td>2(2.4)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>2(2.4)</td>
</tr>
<tr>
<td>No infection</td>
<td>4(4.7)</td>
<td>1(1.18)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>5(5.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>79(92.9)</td>
<td>3(3.5)</td>
<td>1(1.2)</td>
<td>2(2.4)</td>
<td>85(100)</td>
</tr>
</tbody>
</table>

Although it is well known that women are far from achieving equal participation in decision making especially when it comes to leadership and power as evidenced by the fact that only between 10-24 women as at 1995 and 1999 have served as heads of state and government (20, 21). However, from this study, it appears that women in *purdah* fully participate in decision making processes when it comes to reproductive health matters. This could be probably because total loyalty in *purdah* is not expected to the point of death from sickness. This however does not underscore the fact that some husbands in *purdah* do take unilateral decisions on reproductive health issues. In such cases as revealed in the structured interview, altering reproductive health decision making processes in favour of women in *purdah* can best be achieved by educating both these women and their husbands.

The belief that *purdah* system is a problem because it permits up to 3-4 wives to be secluded from taking active part in the social well-being of the community and the country (18) may actually have been misunderstood or misinterpreted by those that practice *purdah*. This is because Pickhall (22) stated that social degradation of women is a crime and a libel on Islam. Pickhall (22) further claims that the un-Islamic Indian style *purdah* system (hijab) which appears to be the type well known is a case of religion overkill. This commandment in the Qu’ran in chapter 33 verse 53 with respect to hijab applies only to the “Mothers of the Believers” (the wives of the holy Prophet, p.b.u.h) but the wordings of the Qur’an in chapter 33 verse 55, applies to all Muslim women in general. There is no screen or (hijab) (*purdah*) mentioned in this verse-it prescribed only a dress (22). This makes the practice of the Indian-style system of *purdah* unlawful.

This work concludes by stating that frantic efforts must be made to educate Muslims in *purdah* so as to ease some restrictions on women activities that affect particularly reproductive health. Promoting the education of Muslim women is a first step in moving beyond the constraints imposed by *purdah*. This struggle appears to be the only tool that will fray the threads in this socially imposed curtain.

**ACKNOWLEDGEMENT**

We thank the Council for the Development of Social Science Research in Africa (CODESRIA) that supported this work with the grant No. 39/T99.

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SIGNIFICANCE OF PYURIA IN THE DIAGNOSIS OF URINARY TRACT INFECTION IN CHILDREN WITH SICKLE CELL ANAEMIA IN MAIDUGURI, NIGERIA

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ABSTRACT

Urinary tract infection (UTI) is a significant cause of morbidity and mortality in children, especially in those with sickle cell disease, who are at higher risk of infections. It will be useful to have a simple test which can be used in resource limited health facilities as a means of screening such children for UTI with the view to instituting prompt treatment. This study is carried out to determine the usefulness of significant pyuria in detecting UTI in febrile children with sickle cell anaemia (SCA). Two hundred and fifty febrile children with sickle cell anaemia that attended State Specialist Hospital and University of Maiduguri Teaching Hospital (UMTH) were prospectively studied with their consent. Urine sample was collected using standard procedure, examined for pus cells and was cultured at the Microbiology laboratory of UMTH. The study showed UTI prevalence of 26%. Significant pyuria was found to have sensitivity of 55.4%, specificity of 77.8%, the efficacy of the test was 72.0% and the test has low positive predictive value of 46.8% in detecting bacteriuria in SCA patients. The significant pyuria observed in this study support its usefulness in the diagnosis of UTI among children with SCA especially in communities having limited facilities or personnel for carrying out urine culture.

Keywords: Sickle Cell Anaemia, Bacteriuria, Pyuria

INTRODUCTION

Urinary tract infection is a significant cause of morbidity in children and those with sickle cell anaemia (SCA) have been reported to be at high risk (1, 2). Diagnosis is often missed in children because symptoms and signs are not overt and usually not necessarily referable to the urinary tract (3). This may be compounded in SCA children who may be having vaso-occlusive crisis involving the abdomen, making it more difficult to differentiate from urinary tract infection (UTI). The prevalence of UTI varies from 0.4% to 7.5% in different childhood populations and there is no age limit for this disease; even newborn are susceptible (4, 5). High prevalence of 6% to 26% has been reported in SCA children (6, 7, 8, 9, 10, 11). Prompt diagnosis of all cases of UTI in children with SCA is highly desirable. Simple test that is sensitive to allow initiation of treatment to relieve symptoms and prevent long term complications of UTI such as renal scarring and other complications is vital (12). There are various methods of diagnosing UTI in children. The gold standard of diagnosis is by culture of clean voided midstream urine or by suprapubic bladder aspiration: A pure growth of >10^5 colony-forming units (CFU) per milliliter of freshly passed urine (13) or any growth from urine that has been obtained by correctly performed suprapubic bladder puncture (14, 15). Transurethral bladder catheterization (TUBC) can also be done to obtain urine for culture (16). The diagnoses of pyuria (leucocyturia) are done on fresh, uncentrifuged / centrifuged urine (17, 18). Quantification of pyuria and its relationship to bacteriuria and presence of symptoms is important in differentiation between colonization and infection (18, 19). A urinary white cell count of ≥10 cells/µl in uncentrifuged urine is taken as significant, while value of ≥5 cells/µl of centrifuged urine is taken as significant (13, 18, 19). There has been a report of pyuria being absent in 50% of patients with significant bacteriuria (18). On the other hand there are numerous other causes of pyuria such as urinary calculi, appendicitis, non infective glomerulonephritis, severe dehydration and chemical injury to the urinary tract (13, 18). Other findings that can be seen in a child with suspected UTI include; haematuria which occurs in one third of children with UTI, proteinuria can also occur in UTI but its absence does not exclude it (14, 15, 18). Urine samples obtained must be processed immediately or preserve in a boric acid solution which preserve pus cells and the bacteria (17). However, this culture could cause delay and the facilities may not be there in most impoverished economies. This will lead to delay in treatment of acutely ill child. Urine microscopy looking for pus cells can provide immediate diagnostic information to enable initiation of treatment. Looking at the techniques and the
resources needed to diagnosed UTI using the gold standard may not be possible in a developing economy like Nigeria that are often confronted with varying degree of challenges ranging from absent or inadequate quality equipment and reagents to unqualified personnel, as well as operational logistics (20, 21, 22). Any method that could be used to solve these problems should be encouraged. It would be useful to have a simple test which can be used in resource limited health facilities as a means of screening such children for UTI with view to instituting prompt treatment. There has been a doubt as to whether assessment of pyuria can be an indicator for infection of urinary tract (19, 23, 24). The use of significant pyuria as a screening test for UTI has not been evaluated recently in our region. Therefore, this study was designed to evaluate the usefulness of significant pyuria in detecting UTI in febrile children with SCA.

MATERIALS AND METHODS

Settings and ethics

This was a prospective and analytical study conducted in the Department of Paediatrics University of Maiduguri Teaching Hospital (UMTH) and State Specialist Hospital Maiduguri between October 2005 and January 2008. Ethical clearance was obtained from the UMTH Ethical Committee. The study site serves as both primary and tertiary level health institution to Borno State and other State in the North Eastern part of Nigeria. It also provides medical services to neighboring countries of Chad, Niger and Cameroon Republics. The study group consisted of two hundred and fifty children aged 6 months to 15yrs that presented with fever and were confirmed to have SCA were enrolled into the study. Consent from parents or guardians and in addition assent from older children were obtained.

Inclusion and exclusion criteria

Patients with SCA aged 6 months to 15 years having axillary temperature of ≥37.5°C and underwent haemoglobin electrophoresis diagnostic test (HBSS) were enrolled into the study. Patients presented with positive history of antibiotic use within one week, children with non infective cause of fever such as tetanus and dehydration and those patients with clinical condition associated with increased risk of UTI (glomerulopathies, obstructive uropathies, diabetes mellitus, severe malnourishment, catheterized patients, autoimmune diseases and malignancies), as well as those patients that decline to give their consent were excluded from this study.

Sampling procedures and measurement of biophysical parameters

Two hundred and fifty consecutive SCA patients based on Singer (24) aged 6 months to 15 years were recruited from the Sickle Cell Clinics of UMTH and State Specialist Hospital (SSH) or while on admission in Emergency Paediatric Units of both Hospitals. Physical examination was directed first at confirming the history of fever, by measuring the temperature (axillary) with a high accuracy LCD (liquid crystal display) Digital Thermometer with a measuring range of 32°C to 44°C. Measurement sensor head was put into the center of the armpit and left in place until there is an alarm from the thermometer. Reading was taken to the nearest 0.01°C. All the patients were weighed appropriately and recorded to the nearest 0.5kg.

Sample collection, transportation and culture

Midstream urine specimen (5 ml) was carefully collected at the time of presentation into 2 sterile universal bottles for each patient that has achieved bladder control. The following steps were performed to minimize the degree of bacterial contamination of the urine; social cleanliness and local disinfection of the meatus and adjacent mucosa with a swab containing eusol solution and dried with sterile gauze, the hand was gloved with autoclaved latex glove and the foreskin in uncircumcised boys was pulled back while in girls spreading of the labia was done to minimize contact of urinary stream with mucosa. For the adolescent girls a trained female resident doctor assisted in collecting some of the specimens. For infants and children who were not toilet trained, suprapubic bladder aspiration (SPA) was used for obtaining the urine specimen under aseptic procedures. The samples were sent immediately to Microbiology Department of the UMTH. The specimen taken at the SSH were kept in a refrigerator at 4°C for period of the clinic (2 – 3 hrs) before being taken to UMTH (10 - 20 minutes drive), where it was processed immediately for microscopy and culture. Pyuria: five milliliters (5 mls) of the urine was centrifuged at the main Microbiology Laboratory of the Teaching Hospital, at 2000 rpm for 5 minutes; a wet preparation was made from the sediment and examined under the microscope at x40 magnification. More than five pus cells per high power field (HPF) were regarded as significant pyuria. Each urine specimen was mixed with the remaining supernatant and inoculated onto blood and MacConkey agar plates and incubated aerobically at 37°C for 48 to 72 hours. A pure colony count of 10⁵ organisms / ml of urine were considered a significant growth. Other sets of culture plates were incubated in carbon dioxide extinction jar at the same temperature for isolation of anaerobes. In case of significant bacteriuria, systematic bacteriology and biochemical tests using standard techniques; catalase, oxidase,
sugar fermentation, motility, urease, citrate, indole, hydrogen sulfide and gas production were carried out based on bacterial gram reactions. Antimicrobial sensitivity test were carried out using modified Kirby-Bauer’s diffusion methods where zones of inhibition were measured (25, 26). Those with positive culture results were treated accordingly.

Data management and data analysis
All the values were expressed as frequency, mean and standard error of the mean. Chi-square test where appropriate was used to determine the level of significance of the categorical variables using computer software SPSS version 16. P-value less than 0.05 were considered significant. Indices to determine the diagnostic usefulness of pus cells in detecting significant bacteriuria was calculated separately using the methods of Galen and Gambino (27).

RESULTS

TABLE 1: AGE AND SEX DISTRIBUTION OF PATIENTS

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number (percentage)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>0.5 – 5.0</td>
<td>78 (31.2)</td>
<td>56 (22.4)</td>
</tr>
<tr>
<td>5.5 – 10.0</td>
<td>39 (15.6)</td>
<td>27 (10.8)</td>
</tr>
<tr>
<td>10.5 – 15.0</td>
<td>28 (11.2)</td>
<td>22 (8.8)</td>
</tr>
<tr>
<td>Total</td>
<td>145 (58.0)</td>
<td>105 (42.0)</td>
</tr>
</tbody>
</table>

Two hundred and fifty febrile children with SCA were studied out of which 145 (58%) were boys while 105 (42%) were girls. The mean age of children was 5.6 ± 4.4 years. The ages of the patients studied ranged from 0.5 to 15 years with largest number of patients falling within the group 0.5 to 5 years (Table 1). The result of this study showed that 161 (64.4%), 113 (45.2%) and 61 (24.4%) had malaria, bone pain crises and suspected UTI as clinical diagnoses at presentation respectively. Other less common clinical diagnoses presented by the patients include; pharyngotonsillitis (18.8%), pneumonia (13.6%), osteomyelitis (5.2%), septicaemia (4.8%), gastroenteritis (2.4%) and otitis media (2.4%) as well as meningitis (1.6%) (Table 2). The frequently isolated organism was Escherichia coli 18 (27.7%) and Klebsiella pneumoniae 16 (24.6%). The least were Coliforms (13.8%) and Salmonella spp (3.1%) (Table 3).

TABLE 2: CLINICAL DIAGNOSIS AMONG PATIENTS*

<table>
<thead>
<tr>
<th>Clinical diagnoses</th>
<th>Number (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>161 (64.4)</td>
</tr>
<tr>
<td>Bone pain crises</td>
<td>113 (45.2)</td>
</tr>
<tr>
<td>Suspected UTI</td>
<td>61 (24.4)</td>
</tr>
<tr>
<td>Pharyngotonsillitis</td>
<td>47 (18.8)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>34 (13.6)</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>13 (5.2)</td>
</tr>
<tr>
<td>Septicaemia</td>
<td>12 (4.8)</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>6 (2.4)</td>
</tr>
<tr>
<td>Otitis media</td>
<td>6 (2.4)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>4 (1.6)</td>
</tr>
</tbody>
</table>

*Some patients had more than one clinical diagnosis
TABLE 3: URINARY BACTERIAL ISOLATES AMONG PATIENTS

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Number of isolate (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>18 (27.7)</td>
</tr>
<tr>
<td><strong>Klebsiella pneumonia</strong></td>
<td>16 (24.6)</td>
</tr>
<tr>
<td><strong>Proteus mirabilis</strong></td>
<td>11 (17.0)</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>9 (13.8)</td>
</tr>
<tr>
<td><strong>Coliforms</strong></td>
<td>9 (13.8)</td>
</tr>
<tr>
<td><strong>Salmonella specie</strong></td>
<td>2 (3.1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>65 (100)</strong></td>
</tr>
</tbody>
</table>

Out of 65 (100%) sickle cell anaemic children with bacteriuria 36 (55.4%) had significant pyuria, while only 41 (22.2%) out of 185 (100%) with no bacteriuria had significant pyuria. The test had sensitivity, specificity and positive predictive values of 55.4%, 77.8% and 46.8% respectively. The efficiency of the test was found to be 72.0%. Pyuria was associated with increased risk of bacteriuria (p<0.001) (Table 4).

TABLE 4: URINE MICROSCOPY (PYURIA) AND CULTURE

<table>
<thead>
<tr>
<th>Pyuria</th>
<th>Urine culture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Significant growth (%)</td>
<td>Insignificant growth (%)</td>
</tr>
<tr>
<td>Significant pyuria</td>
<td>36 (55.4)</td>
<td>41 (22.2)</td>
</tr>
<tr>
<td>Insignificant pyuria</td>
<td>29 (44.6)</td>
<td>144 (77.8)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>65 (100)</strong></td>
<td><strong>185 (100)</strong></td>
</tr>
</tbody>
</table>

p<0.001 (χ²)
Sensitivity = 55.4%
Specificity = 77.8%
Positive predictive value = 46.8%
Negative predictive value = 83.3%

DISCUSSION

Urinary tract infection is common in children as demonstrated in this study. The prevalence of 26% found in this study only confirms how high is UTI in children with SCA, which also agrees with previous studies done within and outside Nigeria (6, 7, 9, 10, 11). This increased risk has been attributed to sludging of sickled cells in the renal vasculature which causes papillary necrosis and loss of urinary concentrating and acidifying ability of the nephrons, resulting in abnormally diluted and alkaline urine which favours bacterial proliferation (28, 29, 30).

In this study significant pyuria or leucocyturia was found to have sensitivity of 55.4% and specificity of 77.8%. The negative and positive predictive values were 83.3% and 46.8% respectively. This implies that to use significant pyuria to diagnose or predict bacteriuria in children with SCA will result in significantly large numbers of false-positive and false-
negative results. Our study with slightly high sensitivity is comparable to that of Smith and colleagues (31) who reported significant pyuria being present in 64% of patient with significant bacteriuria. The specificity in our study was high which also agrees with specificity of 68% reported by Blum et al (32). However, the relatively higher sensitivity of 98% reported by Blum et al (32) compared with 55.4% of the present study is still not clear, but the use of symptomatic women as their sample population for the study may contribute to the observed significant difference. Again, this may show the variability of significant pyuria in detecting bacteriuria in other related studies (33, 34, 35, 36, 37).

Furthermore, the low positive predictive value with relatively higher negative predictive value observed in this study means that relying on this test (pyuria) alone may not be a good predictor of UTI. Therefore, there is a need to look for a more reliable test, otherwise some patients with pyuria and possible UTI would be overlooked and these could be potentially dangerous. It has previously been reported that the risk of renal damage is greater in sickle cell disease (SCD) children (9, 38). Thus early diagnosis and prompt treatment are important and recommended (14).

Interestingly to mention is the findings of other workers among adults and paediatric population irrespective of their haemoglobin genotype status, where they found significance of pyuria as a possible screening method for UTI (31, 32, 39). Although significant pyuria as a method to diagnose UTI or suggest the presence of bacteria appears to be limited in the foregoing study, we still support the view that the test still proves a veritable fall back tool for laboratory diagnosis in rural and some urban communities in our localities and across Africa where laboratory personnel, reagents and equipments for appropriate diagnosis by culture are still lacking (40). In remote areas, it is not practicable to do culture and probably the newer test by strips like nitrite dipstick test (11, 41) or leucocyte esterase dipstick test (33, 42) may not be available. Therefore practitioners can fall back on microscopy of urine for pus cells to tentatively diagnose UTI in SCA children and even non SCA children. Finally, the limitation of pyuria should be factored into final outcome of the test, considering the present study and other referenced studies. Method of urine collection, transportation, storage, degree of precision in the interpretation of either spun or unspun urine specimen (13, 19, 43, 44, 45) are some key factors that needs to be appropriately considered before pyuria become more useful in the diagnoses of UTI.

CONCLUSION

The study has shown that significant pyuria has slightly high sensitivity with apparently high specificity, but low positive predictive value. The efficiency of the test is high as such we recommend for screening purposes in areas where laboratory facilities are inadequate. However, it could still be better that the test be interpreted along with culture results where it is practicable.

ACKNOWLEDGMENTS

We sincerely thank Dr Watila (Head of Sickle cell disease Clinic at the SSH Maiduguri), Dr Hyelazira and Dr Bala of Department of Paediatric, SSH for their immense assistance during collection of samples. We also thank all the Resident Doctors in the Paediatric Haematology / Oncology Unit of UMTH for their technical assistance as well as Laboratory Scientists of the Department of Microbiology of UMTH for their support in the processing of the urine specimen.

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ORIGINAL ARTICLE
COPYRIGHT 2012 AFR. J. CLN. EXPER. MICROBIOL 13(2): 106-111 http://dx.doi.org/10.4314/ajcem.v13i2.9

CAMPYLOBACTER SPP. EPIDEMIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY IN A DEVELOPING COUNTRY, BURKINA FASO (WEST AFRICA)

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RUNNING TITLE: CAMPYLOBACTER IN BURKINA FASO

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ABSTRACT
Data on campylobacteriosis are almost nonexistent in Burkina Faso. In this study conducted from 2006 to 2008 in Ouagadougou, stool specimens and sociodemographic data were collected from 1,246 patients attending the university teaching hospital for enteritis. Stool samples were analyzed for the presence of Campylobacter by the direct culture method on selective mCCDA agar followed by antibiotic susceptibility testing on the isolated strains. The isolation rate of Campylobacter was 2.5%, comprising of the following species C. jejuni (51.8%), C. coli (13.8%), and C. upsaliensis (3.5%). However, 30.9% of the isolates were unidentified. No resistant strain was found to gentamicin. The resistance to amoxicillin+clavulanic acid (3.4%) was lower than those (10.3-34.5%) to the other antibiotics: erythromycin (10.3%), tetracycline (10.3%), ciprofloxacin (13.8%), amoxicillin (24.1%) and ceftriaxone (34.5%). Significant associations were found between Campylobacter enteritis and contact with animals (P<0.003), and HIV infection (P<0.0001), in contrast to other sociodemographic and seasonal factors. From the data obtained Amoxicillin+clavulanic acid appear to be the first choice for treatment. The implementation of a national program may be helpful in controlling the spread of the disease and the increase of resistance to antibiotics.

Keywords: Campylobacter, epidemiology, HIV, drug resistance, Burkina Faso.

INTRODUCTION
Campylobacter gastroenteritis have a major public health importance worldwide, and their rate has been increasing (1, 2). Campylobacter is the most common cause of culture proven bacterial gastroenteritis in developed and developing countries, responsible for 400-500 million cases of diarrhea each year (3). However, the clinical, laboratory and epidemiological data available are mostly from developed countries (1, 2, 4, 5). In these countries, the highest rates are found among children less than five years old and young adults, mostly men aged from 20 to 29. In developing countries where studies have been conducted, the data showed that Campylobacter enteritis is particularly associated with young children (1, 2). These last years, the diarrhea mortality has declined in the world due to increased use of therapies, improved nutrition feeding, hygiene and sanitation. More recent data showed that the rates of campylobac-teriosis have been decreasing in numerous developing countries (3, 6, 7, 8).

Campylobacter infection is a zoonotic disease that can be hyper endemic, linked to outbreaks and sporadic infections. Known risk factors for the disease include ingestion of undercooked poultry and other meat, contaminated food and water or unpasteurized milk and dairy products, direct contact with pets, farm animals, small children, and swimming in lakes, but also travel abroad. Direct person-to-person transmission between adults appears to be uncommon. In some developed countries, seasonal patterns in pets and in human infections as well as travel-related infections have been observed (2, 4, 9, 10, 11). Several hypotheses have been advocated to explain the seasonality of Campylobacter infections, including the controversial role of flies as vectors (12, 13); however much remains to be done about the epidemiology of these pathogens. Clinically, the disease is often more severe and longer in developed countries, with bloody stools, than cases in developing countries where it is shorter and stools are more liquid and can contain leukocytes (1, 13). Campylobacter enteritis can be asymptomatic and self-limiting; in severe cases effective antibiotic therapies are available. However, acquired resistance to macrolides, fluoroquinolones and other antibiotics used most widely gives rise to a challenge in campylobacteriosis control worldwide (3, 14, 15, 16). Data on campylobacteriosis in developing countries including most of the sub-Saharan Africa are few (1, 17, 18, 19). In Burkina Faso, no data of autochthon cases are available for animals, foods, environment or humans. The only scientific report related to Campylobacter enteritis acquired in Burkina Faso is about cases from Belgian (8) and Swedish (9) international travellers who had stayed in the country between 1994 and 2006.
The aim of this study was to present bacteriological and epidemiological data on *Campylobacter* enteritis among autochthon patients with diarrhea in Burkina Faso.

**MATERIALS AND METHODS**

**Patients and Sample collection**
A series of 1246 consecutive specimens of stool were obtained from patients attending clinics at the University Hospital CHUO for acute gastroenteritis, between November 2006 and February 2008. No patient received antibiotic therapy up to 72h prior to the sample collection which was carried out using sterile flasks. Sociodemographic data and macroscopic aspects of stool specimens were recorded using a structured questionnaire. The informed consent of each patient and the approval of the institution ethical committee had been obtained. All stool specimens were collected at the University Hospital and were analysed within 1h after arrival.

**Bacteriological investigations and drug susceptibility testing**

The presence of leukocytes and erythrocytes in stools was determined microscopically. For *Campylobacter* isolation, each sample was streaked onto a modified charcoal cefoperazone desoxycholate agar, mCCDA (Oxoid, United Kingdom). The CCDA comprised of *Campylobacter* blood-free selective agar base with *Campylobacter* selective supplement (Oxoid) and *Campylobacter* growth supplement (Oxoid). The plates were incubated for 48 h at 37°C under microaerophilic conditions (5% O₂, 5% CO₂, 2% H₂ and 88% N₂ by volume) generated by Campy pack plus (Becton Dickinson) or GENbox Microaer (bioMérieux SA; France). Colonies resembling those of *Campylobacter* (gray, flat, and spreading) were further evaluated by Gram’s stain, oxidase and catalase activities, and by growth on duplicate plates of Columbia Blood Agar with 5% (v/v) defibrinated horse blood (bioMérieux SA). The two plates were incubated at 37°C for 48h, under different conditions: one aerobically and one under microaerophilic conditions. Oxidase- and catalase-positive colonies exhibiting a characteristic Gram stain appearance (Gram-negative S-shaped rods) and growing only under microaerophilic conditions were reported to identify the genus of *Campylobacter*. Suspicions of these colonies to a turbidity equivalent to 6 McFarland were inoculated to API® Campy gallery system (bioMérieux) to differentiate the *Campylobacter* strains into species.

Drug susceptibility tests of the isolates were conducted by disc diffusion method: all laboratory-confirmed *Campylobacter* isolates were inoculated onto Mueller-Hinton agar plates supplemented with 5% sheep’s blood (bioMérieux) and incubated at 37°C for 24h. Amoxicillin (25µg), amoxillin/clavulanic acid (10µg), ceftriaxone (30µg), gentamicin (10µg), erythromycin (15µg), tetracycline (30µg), nalidixic acid (30µg) and ciprofloxacin (5µg) disks (bioMérieux) were tested. The disc diffusion method was used, according to the guidelines of the French Society for Microbiology (20). In this study any strain with an intermediate sensitivity was considered resistant. *C. jejuni* ATCC 25936 and *C. coli* ATCC 33559 strains were used as controls throughout the testing period.

**Statistical analysis**
The Epi-Info 3.3.2 version 2004 software was used to record all data. Comparisons between qualitative variables were done using the Chi-2 test (Likelihood Ratio or Linear-by-Linear Association or Mantel-Haenszel method). Statistical significance between differences was set at P<0.05.

**RESULTS**

**Patient characteristics**
Among the 1246 patients, 638 (51.2%) were male. Their mean age was 21 years [ranges: 0-80years]; the most important age ranges were 0-5 years (n=494; 39.7%), 21-25 years (n=174; 11.9%) and 26-30 years (n=158; 10.9%). Patients living in Ouagadougou (n=1188; 95.3%) were more represented than those coming from other urban and rural areas (n=58; 4.7%). More than half (n=670; 53.8%) of overall patients were educated and those hospitalized (n=640; 51.4%) were more represented than outpatients (n=606; 48.6%). Seven (0.6%) patients were HIV-1 positive.

**Macroscopic aspects of stool specimens and faecal leukocytes**
Four types of stools were reported: pasty (65%), liquid (18.9%), mucous (11.1%) and formed (5%). Pasty stool specimens were more frequent than other types. Leukocytes were found in 164 (13.2%) fresh stool specimens after microscopic examination.

**Bacteriological data**
In this study in Burkina Faso, the isolation rate of *Campylobacter* was 2.3% (29/1246). *C. jejuni* (51.8%, comprising 38% of *C. jejuni jejuni* and 13.8% of *C. jejuni doylei*) and *C. coli* (13.8%) were the most prevalent species. However, 30.9% (n=39) of strains had *Campylobacter*’s characteristics, even if they could not be identified using Api® Campy (bioMérieux SA).

The results of drug susceptibility tests are reported in Table 1. They showed that only gentamicin was effective against all strains. Ceftriaxone- and amoxicillin-resistant strains represented 34.5% and 24.1%, respectively. The resistant rate was moderate to erythromycin (10.3%) and tetracycline (10.3%), and low to amoxicillin/clavulanic acid (3.4%).

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**TABLE 1: SUSCEPTIBILITY OF CAMPYLOBACTER STRAINS TO ANTIBIOTICS**

<table>
<thead>
<tr>
<th>Antibiotic tested</th>
<th>Sensitive (%)</th>
<th>Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (AM)</td>
<td>22 (75.9)</td>
<td>7 (24.1)</td>
</tr>
<tr>
<td>Amoxicillin+clavulanic acid (AMC)</td>
<td>28 (96.6)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>19 (65.5)</td>
<td>10 (34.5)</td>
</tr>
<tr>
<td>Gentamicin (GM)</td>
<td>29 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin (E)</td>
<td>26 (89.7)</td>
<td>3 (10.3)</td>
</tr>
<tr>
<td>Tetracycline (TE)</td>
<td>26 (89.7)</td>
<td>3 (10.3)</td>
</tr>
<tr>
<td>Nalidixic acid (NA)</td>
<td>19 (65.9)</td>
<td>10 (34.1)</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>25 (86.2)</td>
<td>4 (13.8)</td>
</tr>
</tbody>
</table>

Clinical illness of patients infected by *Campylobacter*

Clinical signs of the patients on presentation were as follow: diarrhea (62.1%), abdominal pains (62.1%), fever (51.7%), asthenia (44.8%) and vomiting (31%) were the most common symptoms. A single (3.4%) infected patient had none of these signs. Three of 7 (43%) of HIV-positive patients had *Campylobacter*, but only 26 (2%) among the other 1239 patients.

**Epidemiological characteristics of Campylobacter enteritis**

The patient variables studied are reported in Table 2. The number of *Campylobacter* strains isolated from females was more prominent, but the difference was not statistically significant ($P=0.15$; Odds Ratio: 0.575; 95%CI: 0.26-1.22). No statistically significant difference was found between all age ranges of *Campylobacter* infected patients ($P=0.14$) except those of 0-5 and 21-40 years ($P=0.62$; OR: 0.823; 95%CI: 0.37-1.79]) which showed a higher infection frequency. Attendance at school or not was not a risk factor for *Campylobacter* enteritis ($P=0.69$; OR: 1.158; 95%CI: 0.55-2.42); no difference was associated with the level of education. All the cases of *Campylobacter* infections were found in patients from Ouagadougou. The patients who were in touch with pets (3.4%) were more frequently infected than those who had no contact (2%) with animals ($P=0.03$; OR: 4.23; 95%CI: 0.19-9.93).

The frequencies of isolation of *Campylobacter* were more common in January, June, July, October and November than other months in 2007 (Figure 2). The complete data for the year 2007 did not show any statistically significant difference in the monthly rates of infection ($P=0.20$). There was no difference between the rates of infection during the dry season and the rainy season ($P=0.85$; OR: 0.930; 95%CI: 0.42-2.02); indicating that, there was no seasonality in the pattern of the disease.

**DISCUSSION**

The rate of *Campylobacter* enteritis in the studied population was 2.3%. Such a rate in urban sites of Africa is uncommon: the rates reported in most of other developing countries are usually higher (1, 3, 19). However, a comparable rate of 2.4% was reported in the Northeast of Brazil (21) and in Teheran (22). *C. jejuni* and *C. coli* were the main species identified in Burkina Faso: this result was comparable to those found as well in other developing countries where enteritis due to *Campylobacter* have been studied as in developed countries (1, 3, 5, 6, 19, 23, 24).

More than 30% of the strains in Burkina Faso were not identified by the gallery Api60Campy: this limitation has also been reported in was reported by other studies using this gallery. The authors reported misidentifications of *C. concisus* for *C. mucosalis*, and they reported additional problems in identifying certain *C. coli* and *C. lari* strains (4, 25). The use of molecular techniques will allow these strains to be typed and possibly identify new pathogenic species of *Campylobacter*.

The infection rate in females was higher than in males, although this difference was not statistically significant. In some developed countries, it has been demonstrated that the rate of *Campylobacter* enteritis was higher in males than in females (6, 23, 26). The association between gender and campylobacteriosis may vary according to the behaviour, geographical area and the population, and it can be different within the same country. In Burkina Faso, children of 0-5 years old and young adults of 21-40 years old were the most infected. The high recovery rate of *Campylobacter* species in children is common worldwide, and Burkina Faso is no exception. However, a high rate in young adults comparable to that in children can be associated with a common source of contamination: indeed, these young adults in Burkina Faso are workers who eat in the street, outside of their house. Neither schooling, nor the level of education were risk factors to enteritis due to *Campylobacter*, contrary to data reported in a study in Jordan where education was a risk factor that correlated significantly with diarrhea due to *Campylobacter* enteritis (27). The education of the consumers of vector products of campylobacteriosis is important in the implementation of national programs of surveillance and fight against *Campylobacter*. More
### TABLE 2: SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Campylobacter Positive</th>
<th>Total</th>
<th>P value</th>
<th>OR</th>
<th>95%CI</th>
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<td>1246</td>
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<td>494</td>
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<td>122</td>
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<td>463</td>
<td>477</td>
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<td>Education level</td>
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<td>Residence in Burkina</td>
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</table>

more than 95% of the patients came from Ouagadougou and the others were also city-dwellers: these findings are comparable to those reported by Beatty et al., (28) in Kenya. The close proximity to animals in developing countries contributes to easy and frequent acquisition of Campylobacter which can be
transmitted to humans directly by infected animals (1, 3, 4, 5). In Burkina Faso, the results showed that contact with live animals was a significant risk factor (OR: 0.423; 95% CI: 0.19-0.93; P=0.03). The prevalence of HIV infection in Burkina Faso was 1.6% in 2007, with an estimated number of 130,000 [110,000-160,000] people of all ages living with HIV (29). The diarrheic diseases constitute an important part of the opportunistic infections in AIDS-patients. Statistically significant associations between Campylobacter enteritis and AIDS have been reported (1, 30). The clinical manifestations and substantial mortality are more frequent in AIDS patients than HIV-negative patients (1, 24, 31). The results obtained in Burkina Faso confirmed these observations: HIV-positive patients were more infected by Campylobacter than HIV-negative patients (P<0.0001). However, Awol et al., (32) have reported higher prevalence rates of Campylobacter species in HIV-negative patients (25%) than in HIV-positive (13.1%). Manfredi et al., (33) agree with such a finding in the era of highly active antiretroviral therapy (HAART): HIV-infected persons progressively feel better and clinically they look the same as other patients in the general population. Numerous works reported that diarrheas due to Campylobacter are clinically less severe in the developing countries than in the developed countries. Watery, mucosal and/or even bloody stools associated with fever, abdominal pain, vomiting and presence of fecal leukocytes were described (1, 3, 5, 21, 22). In Burkina Faso, no bloody stools were found and mucosal stools were few (7%) in Campylobacter infected patients. The pasty stools (62.1%), abdominal pain (62.1%), diarrhea (51.7%), fever, asthenia (44.8%) and vomiting (31%) were the main clinical signs found in infected patients. The presence of leukocytes in 13.2% of stools could be associated with other invasive bacteria often found in co-infections. No seasonality was found with Campylobacter enteritis in Burkina Faso which is similar to reports from several developing countries (1, 2, 5); these findings were different from data in Ifakara (Tanzania), were Vargas et al., (34) found Campylobacter species in 2.5% of children less than five years of age, only in the dry season. However, the seasonality of campylobacteriosis is much debated (10).

Several studies have reported good correlations between disc diffusion and agar dilution methods for the drug susceptibility testing of Campylobacter in developed and in developing countries (4, 15, 35, 36). In developing countries, various patterns of Campylobacter susceptibility to antibiotics were described. The disc diffusion method was used in the study in Burkina Faso. The rate of resistance to amoxicillin (24.1%) was reduced to amoxicillin+clavulanic acid (3.4%), while it was with ceftriaxone. These antibiotics are commonly used against Gram-negative rods in developing countries. The amoxicillin and ampicillin activities against bacteria are comparable usually. Recent data on the resistance of Campylobacter strains isolated from humans to amoxicillin are rare.

The rate of resistance to amoxicillin+clavulanic acid in the study in Burkina Faso was lower than that (44%) reported by Samie et al., (37) in South Africa. However, the resistance rate to ceftriaxon in Burkina Faso was higher that (8%) in South Africa while it was lower than those reported in other cities from developing countries as Ilorin in Nigeria (84%) or Teheran in Iran (47%) (23, 38). Gentamicin was the sole effective antibiotic against all the strains of Campylobacter in Burkina Faso as in Iran (38); resistance of 10.2% was reported from north India (39), 4 to 21% in Nigeria (23, 37) were found to this antibiotic. Erythromycin is widely used in both children and adults world wide. It has been reported that the resistance rates of Campylobacter to erythromycin is increasing and vary between 12 and 95% (28, 37, 40); however, the rate was lower in Burkina Faso (10.3%) and such lower rates were also found in north India, (6.1%) and in Sudan (1.7%) (15, 39). Besides, Serichantalergs et al., (16) reported a decrease in the Campylobacter resistance rates to erythromycin, from 1996 to 2000, in urban Bangkok (Thailand). None resistant strain was found in studies conducted in Kampala (Uganda) and in Ilorin (Nigeria) (23, 41). The rate of Campylobacter resistance to tetracycline was 10.3%: with this antibiotic also various resistance patterns are found in developing countries (15, 28, 37, 38, 39). All the studies in developing countries report resistant Campylobacter to nalidixic acid, at various rates, but always higher than those to ciprofloxacin. In a few countries, probably in limited populations, ciprofloxacin was reported as effective against all the strains of Campylobacter (23, 31). The rate may be lower than 10% in other developing countries, (41). However, the Campylobacter resistance to fluoroquinolones is high (11-95%) and continue to increase in developing countries, globally, particularly in Thailand (16, 28, 37).

The data reported in Burkina Faso (Table 2) is in keeping with this trend. The findings in the study in Burkina Faso showed that contact with infected animals and HIV infection are significant risk factors for acquiring Campylobacter enteritis. No seasonality was associated with the disease. The strain susceptibility testing to antibiotics showed various patterns; gentamicin was effective against all the strains, but amoxicillin+clavulanic acid, erythromycin and tetracycline showed satisfactory activities allowing for their use against Campylobacter enteritis in Burkina Faso. The implementation of a national program to fight against campylobacteriosis will allow for a better understanding of the epidemiology of these bacteria and to survey the evolution of their resistance to antibiotics. The use of molecular techniques will enhance the identification and estimation of the variety of the species of Campylobacter in the Burkina Faso.
ACKNOWLEDGMENTS

We acknowledge medical and laboratory staffs at the University Hospital CHUYO for the commitment in this study. We acknowledge also Dr Musa Hassan-King PhD, MSc (Medical Research Council, Gambia; MVP Technical Advisor) for accepting to review the manuscript and the team of Dr SZ in Heidelberg for their support.

REFERENCES


EPIDEMIOLOGICAL STUDIES ON PROTEEAE ISOLATES FROM CLINICAL SPECIMENS IN THE LAQUINTINIE HOSPITAL IN DOUALA, CAMEROON


Faculty of Health Sciences, University of Buea, Republic of Cameroon

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Abstract

The tribe Proteae is a group of bacteria within the family Enterobacteriaceae and is responsible for most cases of nosocomial infections in hospital settings. The objective of this study was to determine the prevalence of members of Proteae from clinical specimens in Laquentinie hospital in Douala. Specimens were collected from patients and screened for Proteae using standard microbiological and biochemical methods (API 20 Enterobacteriaceae gallery). Of the 3414 clinical specimens made of 2712 urine, 264 blood, 243 CSF and 195 wounds and burns, 1136 (33.3%) were wounds and burns, 10 (6.1%) from blood and 7 (4.3%) from CSF. Speciation of the Proteae isolates showed that 111 (14.4%) isolates were identified as members of Proteae of which 110 (67.1%) were from urine, 37 (22.6%) from wounds and burns, 10 (6.1%) from blood and 7 (4.3%) from CSF. Speciation of the Proteae isolates showed that 111 (67.7%) were Proteus mirabilis, 21 (12.8%) Proteus vulgaris, 11 (6.7%) Providencia alcalfaciens, 6 (3.6%) Providencia stuartii, 4 (2.4%) Morganella morganii and 5 (3.0%) Proteus penneri and Providencia rettgeri. There was a significant difference between the type of clinical specimens and the age of patients ($X^2 = 52.623, p < 0.05$). Most Proteae isolates where susceptible to imipemen, ceftazidine, chloramphenicol, gentamicin, nalidixic acid, ofloxacin and amikacin. These findings have significant clinical and epidemiological implications.

Keywords: Proteae isolates, Clinical specimens, Laquentinie Hospital, Cameroon

INTRODUCTION

The tribe Proteae consists of a unique group of bacteria within the family Enterobacteriaceae. The genera include; The Enterobacteriaceae are Gram-negative, non-sporing rods measuring about 1-4 $\times$ 0.6µm. They are peritrichous and motile. Proteae can be found in animals, soil and in the intestinal tract of humans. The Proteae which include Proteus, Providencia and Morganella are unique among the Enterobacteriaceae in that, they have the ability to oxidatively deaminate a wide range of amino acids especially phenylalanine. Proteus, Providencia and Morganella are all oxidase negative and urease positive for some of their strains. They are readily isolated from pathological specimens [1]. Proteae are of concern in that they have been involved in recent years with human infections, especially in the hospital areas. They have been readily isolated from urinary tract infections especially in patients with indwelling urinary catheters. They are thought to be responsible for almost 10% of nosocomial infections [2,3]. In addition, a number of Proteae are often resistant to antibiotics commonly used in hospital practice and as such may facilitate their spread within this environment. Mistry et al. [4] described high prevalence of Proteus mirabilis, in skin abscesses of the maxilla, which had potential implications for selection of antimicrobial therapy. A case of suppurrative pericarditis caused by Proteus mirabilis and Citrobacter diversus were also identified [5] even though polymicrobial gram-negative infections in cases of pericarditis are known to be rare. Morganella morganii was found to be the sole potentially pathogenic bacterial species present in the feces of some patients with diarrhea. Nosocomial infections outbreaks involving some strains of M. morganii are rare but are associated with serious morbidity and high mortality [1]. M. morganii has also been isolated from a patient with diabetic foot ulcer which resulted to a gas gangrene which was confounded with that caused by Clostridium [6]. M. morganii has also been responsible for the death of a 17 days termed neonate. Usually, it does not cause perinatal infection but will do in the immunocompromised patients and premature infants [7]. Providencia species occur in normal feaces and has been isolated from epidemic and sporadic cause of diarrhea in man though their importance in the causation of diarrhoeal disease
is not easy to assess. However, *Providencia rettgeri* has been associated in cases of traveller’s diarrhea in Japan [8]. They also cause UIT especially *Prv. stuartii* and *Prv. rettgeri* which are often hospital acquired [9]. Additionally, *Pro. alcalifaciens* has been reported in food borne infections outbreaks [10]. Generally, *Providencia alcalifaciens* is associated with infections of the intestinal tract and *Providencia stuartii* and *Pro. rettgeri* with infections of the urinary tract although exceptions do occur [1].

Epidemiological data on Proteae isolates in clinical specimens and their antimicrobial sensitivity testing is important to help clinicians in the empirical selection of medications. In Cameroon, such data are scarce in healthcare settings due to dwindling resources and information used is usually from developed countries. The objective of this study was therefore to determine the prevalence of infections caused by organisms of the tribe *Proteae* from clinical specimens in Douala Laquintinie hospital.

**MATERIALS AND METHODS**

**Study design and subjects**
This study was conducted in the Douala Laquintinie hospital, Cameroon, a tertiary health care hospital providing a full range of surgical, medical and super speciality facilities. The study was carried out from January to June 2010. All the subjects were patients sent to the bacteriology laboratory by physicians. There was no formal definition and recruitment was at the discretion of the attending physicians. However, prior to specimen collection, patients were explained the purpose of the study and made to understand that it was not a hospital obligation to participate in the research and neither was it a pre-requisite to accessing any hospital services publicly available. Patients’ consent to participate was solicited. Ethical clearance was obtained from the Regional Delegation of Public Health.

**Bacteriological analysis**
Smears of the specimens were made and Gram-staining was also done to have an idea of the expected bacteria be it rod or cocci. For the urine samples, wet mount was made using the Mallassez counting chamber. Five bands were counted and multiplied by two to get the number of pus cells per cubic millimeter (mm³). Specimens that had less than 5 pus cells/mm³ cells were not cultured and those above were cultured. Specimens were plated on cysteine-lactose-electrolyte-deficient (CLED) agar, Eosin-methylene blue (EMB) and blood agar. After overnight incubation at 37°C, a representative of each type of oxidase Negative Non-lactose fermenting colony from the EMB and CLED agar were subcultured into peptone water and incubated at 37°C for a few hours. These were further inoculated on phenylalanine and urease agar slants and incubated overnight at 37°C. Proteae isolates changed the phenylalanine agar to green. Further test were carried out for full identification. For blood Culture, about 10ml of patient’s blood was added to Brain Heart Infusion Broth (BHI). The broth was incubated at 37°C and observed for growth on the 1st, 2nd, 3rd, 5th, 8th, 16th and 21st day. When positive growth was observed the broth was subcultured into blood and EMB agar.

All cultured plates were observed for colonial morphology of the bacteria such as size on agar, blood agar plates were used to detect swarming and hemolysis. The characteristic smell of Proteae isolates was also noted. Oxidase test were performed on the non-lactose fermenting colonies using filter paper impregnated with a freshly prepare 10% solution of tetramethyl-p-phenylene-diamine dihydrochloride. Proteae isolates were purified alternatively on EMB agar before preservation. Purified colonies were maintained on nutrient agar slants at 4°C until used. Isolates were identified by their biochemical characteristics using the API 20 Enterobacteriaceae gallery (Biomérieux, France) as described by Cheesbrough[11].

The sensitivity of each identification test medium was confirmed by inoculating one or two uninoculated tubes of the batch alloy with the test medium inoculated with positive and negative control organisms. For the positive control, one of the test medium was inoculated with a stock culture of a bacterium (e.g a phenylalanine deaminase positive Proteus species) to give a positive reaction and another tube with stock culture known to give a negative reaction (e.g *Escherichia coli* ). These positive and negative controls were incubated at 37°C and examined along site the alloy.

**Antibiotic susceptibility testing**
Single antibiotic impregnated disk were used with the following concentrations; amikacin (10µg), imipenem (10µg), ciprofloxacin (5µg), clotrimoxazole (30µg), tobramycin (30µg), chloramphenicol (30µg), cefazidime (10µg), gentamicin (10µg), amoxycillin (30µg), ofloxacin (10µg), and nalidixic acid (30µg). Four to five colonies of identical morphology were emulsified into 3-4ml of sterile distilled water. The inoculum was prepared to meet the 0.5 McFarland standard (approximately 10⁵ microorganism per ml), (PML Microbiologicals, Inc, 2001). The prepared Mueller-Hinton agar plates were inoculated by streaking method using a swab of
the inoculums. The plates were allowed to dry for 5 minutes to remove excess moisture. The antibiotic discs were then firmly placed on the inoculated plates using a flame sterile forceps. The discs were evenly distributed to allow an edge not less than 15 mm from the walls of the petridish. The inoculated plates were inverted and incubated aerobically at 37°C for 24 hours. After overnight incubation, each plate was examined and the diameter of the complete inhibition zones were noted and measured with a caliper in millimeter. The inhibition zones were interpreted using the manufacturer's standard table as being; susceptible (sensitive), intermediate or resistant.

A control test was done for the cultures since disc diffusion test vary with a number of experimental conditions and the organism in question. The control culture (E. coli) was plated simultaneously with the test culture and on separate plates. The zones of inhibition produced in the cultures by the same discs were compared.

Statistical analysis

Chi-square and t-test were used to test for the significance of the results obtained. p<0.05 was considered statistically significant.

RESULTS

All Proteae isolates were phenylalanine positive. Only the Proteus isolates swarmed on blood and EMB agar at 37°C though a few strains did not. Of the urease positive isolates, the test results could be easily observed within 6 hours. Only P. mirabilis were indole negative. Some P. vulgaris strains were salcin and esculin positive. All isolates were oxidase negative, non-lactose fermenting colonies and gram negative rods.

A total of 3414 specimens were received from the hospital service areas of; Medical, Surgery, Pediatrics, Out-patients, Obstetrics and Gynaecology. Distribution of the clinical specimen by service area is shown in Table 1. The largest number of wounds and burns were from the surgery wards, while most urine samples were from the out-patients and pediatrics services. There was no blood and cerebrospinal fluid (CSF) samples from obstetrics and gynaecology services.

<table>
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<th>Wounds and burns</th>
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<td>17</td>
<td>3</td>
<td>41</td>
</tr>
<tr>
<td>Medicine</td>
<td>Samples</td>
<td>360</td>
<td>102</td>
<td>36</td>
<td>75</td>
<td>573</td>
</tr>
<tr>
<td></td>
<td>Bacterial isolates</td>
<td>177</td>
<td>36</td>
<td>12</td>
<td>18</td>
<td>243</td>
</tr>
<tr>
<td></td>
<td>Proteae</td>
<td>36</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Pediatrics</td>
<td>Samples</td>
<td>726</td>
<td>126</td>
<td>6</td>
<td>108</td>
<td>966</td>
</tr>
<tr>
<td></td>
<td>Bacterial isolates</td>
<td>324</td>
<td>12</td>
<td>5</td>
<td>20</td>
<td>361</td>
</tr>
<tr>
<td></td>
<td>Proteae</td>
<td>36</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>Obstetrics and Gynaecology</td>
<td>Samples</td>
<td>150</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>Bacterial isolates</td>
<td>27</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Proteae</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Out –patients</td>
<td>Samples</td>
<td>1419</td>
<td>24</td>
<td>45</td>
<td>51</td>
<td>1539</td>
</tr>
<tr>
<td></td>
<td>Bacterial isolates</td>
<td>339</td>
<td>3</td>
<td>30</td>
<td>3</td>
<td>375</td>
</tr>
<tr>
<td></td>
<td>Proteae</td>
<td>12</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2712</td>
<td>264</td>
<td>195</td>
<td>243</td>
<td>3414</td>
</tr>
</tbody>
</table>

Table 2 shows the distribution of bacterial isolates by clinical source. There were 1136 bacterial isolates of which 230 (20.2%) were Gram positive and 906 (79.7%) were Gram negative. Of the Gram negative bacteria, 164 (18.1%) were Proteae isolates. The greatest number of bacterial isolates was from urine followed by wounds and burns. Staphylococcus aureus accounted for 114 (49.6%) of the gram positive isolates. Most of the 18.1% Proteae were from urine followed by wounds and burns then blood specimens. There was a significant difference between the bacterial species and the site of recovery, (X² = 396.79  p<0.005).
Table 2: Clinical specimens' source and distribution of bacterial isolates

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>No. of isolates</th>
<th>No. of bacteria isolates from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wounds and burns</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>114</td>
<td>11</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>51</td>
<td>6</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>156</td>
<td>12</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>453</td>
<td>18</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>39</td>
<td>6</td>
</tr>
<tr>
<td>Proteae</td>
<td>164</td>
<td>37</td>
</tr>
<tr>
<td>Uncharacterized species</td>
<td>71</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>1136</td>
<td>129</td>
</tr>
</tbody>
</table>

\[ X^2 = 396.79 \quad p < 0.005 \]

Table 3 shows the frequency of isolation of Proteaeae species from clinical specimens. Of the 164 Proteaeae isolates, 111 (67.7%) were made of *P. mirabilis* 21(12.8%) of *P. vulgaris* 11 (6.7%) of *Providencia. alcalifaciens*, 6 (3.6%) of *Pro. stuartii*, 4 (2.4%) of Morganella morganii and 5 (3.0%) of *P. penneri* and *Prv. rettgeri* respectively. There was no significant difference between the species of Proteaeae isolated and the clinical specimens (\(X^2=17.63, p>0.05\)). The Distribution of Proteaeae isolates by age in relation to clinical source is shown in Table 4. A greater number of Proteaeae isolates were from the elderly aged 50 and above followed by the younger aged 0 – 10 years, (\(X^2 = 52.623 \quad p<0.05\)).

Table 3: Frequency of isolation of Proteaeae species from clinical specimens

<table>
<thead>
<tr>
<th>Source</th>
<th>No of positive bacteria isolates</th>
<th>No (%) of Proteaeae isolates</th>
<th>Proteaeae isolates identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. mirabilis</em></td>
</tr>
<tr>
<td>Wounds and burns</td>
<td>129</td>
<td>37 (28.7)</td>
<td>22</td>
</tr>
<tr>
<td>Urine</td>
<td>906</td>
<td>110 (12.1)</td>
<td>78</td>
</tr>
<tr>
<td>Blood</td>
<td>54</td>
<td>10 (18.5)</td>
<td>7</td>
</tr>
<tr>
<td>CSF</td>
<td>47</td>
<td>7 (14.9)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>1136</td>
<td>164 (14.4)</td>
<td>111</td>
</tr>
</tbody>
</table>

\[ X^2 = 17.63 \quad p>0.05 \]

Table 4: Distribution of Proteaeae isolates by age and clinical specimens

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Wounds and burns</th>
<th>Blood</th>
<th>Urine</th>
<th>CSF</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10</td>
<td>4</td>
<td>9</td>
<td>55</td>
<td>6</td>
<td>74</td>
</tr>
<tr>
<td>11 - 20</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>21 - 30</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>31 - 40</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>41 - 50</td>
<td>8</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>10</td>
<td>0</td>
<td>34</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>10</td>
<td>110</td>
<td>7</td>
<td>164</td>
</tr>
</tbody>
</table>

\[ X^2 = 52.623 \quad p<0.05 \]

Table 5 shows the distribution of Proteaeae species isolates by age of infected patients. In all, *P. mirabilis* accounted for 111 (67.7%) of Proteaeae species isolated. There was a significant level of association between age of patients and species distribution (\(X^2=47.13 \quad p<0.05\)).

P. *penneri* isolates were recovered mostly from the patients aged 0 – 10 years. A greater number of *Prv. rettgeri* isolates were in the infected patients of age greater than 50.
**TABLE 5: DISTRIBUTION OF PROTEAE SPECIES ISOLATES BY AGE OF PATIENTS**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No of isolates</th>
<th>P. mirabilis(%)</th>
<th>P. vulgaris</th>
<th>P. penneri</th>
<th>Prev. stuartii</th>
<th>Prev. alcalifaciens</th>
<th>Prev. rettgeri</th>
<th>M. morganii</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>74</td>
<td>48 (64.9)</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11-20</td>
<td>9</td>
<td>4 (44.4)</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21-30</td>
<td>9</td>
<td>3 (33.3)</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>2 (50)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>41-50</td>
<td>23</td>
<td>15 (65.2)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>45</td>
<td>39 (86.7)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>164</td>
<td>111</td>
<td>22</td>
<td>5</td>
<td>6</td>
<td>11</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

\[X^2 = 47.13 \quad p<0.05\]

Table 6 shows the frequency of Proteae isolation from UTI's by service areas. From the surgery service, 20 (51.3%) of Proteae isolates caused urinary tract infections. Less Proteae UTI's were noted in out-patients 12 (3.5%) there were no marked significance of UTI's caused by Proteae with respect to those caused by other bacteria in relation to various service areas \((t=1.43\quad p>0.05)\).

**TABLE 6: FREQUENCY OF PROTEAE ISOLATION FROM URINARY TRACT INFECTIONS BY MEDICAL SERVICE AREA**

<table>
<thead>
<tr>
<th>Service</th>
<th>No of bacterial UTI cases</th>
<th>No (%) of Proteae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>39</td>
<td>20 (51.3)</td>
</tr>
<tr>
<td>Medicine</td>
<td>177</td>
<td>36 (20.3)</td>
</tr>
<tr>
<td>Paediatrics</td>
<td>324</td>
<td>36 (11.1)</td>
</tr>
<tr>
<td>Obstetrics and Gynaecology</td>
<td>27</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Out – patients</td>
<td>339</td>
<td>12 (3.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>906</strong></td>
<td><strong>110 (12.1)</strong></td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the listing of gram-negative bacteria responsible for nosocomial infections, members of the tribe Proteae are inclusive. The presence of Proteae in clinical specimen is of great interest since like other Enterobacteriaceae they are opportunistic and may cause morbidity and motility [1].

In this study, gram-negative bacteria accounted for 79.7% of all isolates from clinical specimens examined and cultures in the medical microbiology laboratory. Gaynes and Edwards [12] in their surveillance study on nosocomial infections caused by gram-negative bacteria in intensive care units from a study period of 1986 to 2003, found that gram negative bacteria caused 23.8% of blood stream infections, 33.8% of surgical site infections and 71.1% of urinary tract infections.

Clinical findings from this research also showed that after E. coli, Proteae were the second group of bacteria recovered in this study. E. coli being the major bacteria species isolated is probably due to the existence of E. coli as a normal intestinal flora making its colonization of other parts of the body easier especially in cases of immune suppression. This is in agreement with previous findings [13] that E. coli accounts for the largest cause of cystitis, prostates and pyelonephritis in hospitals acquired infections. In another study [1], Proteus ranked third as the case of nosocomial infections in hospitals. From another study by De Champ et al.[14] Proteus ranked second after E. coli among enterobacteria responsible for nosocomial infections. In the present study, members of the tribe Proteae were mostly recovered from urine samples. They have always been recognized as important cause of nosocomial urinary tract infections associated with long-term catheterization [13].
### TABLE 7: PATTERN OF ANTIMICROBIAL RESISTANCE AMONG PROTEEAE ISOLATES

<table>
<thead>
<tr>
<th>Proteae species</th>
<th>TSU</th>
<th>GEN</th>
<th>AMK</th>
<th>TOB</th>
<th>NAL</th>
<th>OFX</th>
<th>CIP</th>
<th>AMO</th>
<th>IMI</th>
<th>CAZ</th>
<th>CMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. mirabilis (111)</td>
<td>72 (65)</td>
<td>33 (30)</td>
<td>15 (13)</td>
<td>80 (72)</td>
<td>27 (24)</td>
<td>30 (27)</td>
<td>47 (42)</td>
<td>43 (39)</td>
<td>13 (12)</td>
<td>63 (57)</td>
<td>68 (61)</td>
</tr>
<tr>
<td>P. vulgaris (21)</td>
<td>12 (57)</td>
<td>12 (57)</td>
<td>8 (26)</td>
<td>9 (42)</td>
<td>15 (71)</td>
<td>16 (76)</td>
<td>15 (71)</td>
<td>21 (100)</td>
<td>3 (14)</td>
<td>4 (19)</td>
<td>19 (90)</td>
</tr>
<tr>
<td>P. penneri (5)</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (80)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>5 (100)</td>
<td>3 (60)</td>
<td>1 (20)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Prev. stuartii (6)</td>
<td>1 (17)</td>
<td>6 (100)</td>
<td>1 (17)</td>
<td>6 (100)</td>
<td>4 (67)</td>
<td>3 (50)</td>
<td>2 (33)</td>
<td>0 (0)</td>
<td>3 (50)</td>
<td>2 (33)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Prev. alcalifaciens (11)</td>
<td>9 (82)</td>
<td>6 (55)</td>
<td>10 (91)</td>
<td>8 (73)</td>
<td>7 (64)</td>
<td>7 (64)</td>
<td>9 (82)</td>
<td>11 (100)</td>
<td>3 (27)</td>
<td>3 (27)</td>
<td>10 (91)</td>
</tr>
<tr>
<td>Prev. rettgeri (5)</td>
<td>1 (20)</td>
<td>4 (80)</td>
<td>4 (80)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (80)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>2 (40)</td>
<td>0 (0)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>M. morganii (4)</td>
<td>4 (100)</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>4 (100)</td>
<td>4 (100)</td>
<td>2 (50)</td>
<td>4 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (100)</td>
<td></td>
</tr>
</tbody>
</table>

\(X^2 = 117.12\) \(p<0.005\)

AMK, Amikacin; IMI, Imipenem; CIP, Ciprofloxacin; TSU, Clotrimoxazole; TOB, Tobramycin; CMP, Chloramphenicol; CAZ, Ceftazidime; GEN, Gentamicin; AMO, Amoxycillin; OFX, Ofloxacin; NAL, Nalidixic acid.

The predominance of Proteus among all the Proteae is consistent with previous reports [1, 14]. Apart from urine specimens being the source of most clinical isolates of Proteae, others were recovered from wounds and burns, followed by blood and the least in CSF. P. mirabilis was recovered more often than P. vulgaris especially in UTIs. The predominance of P. mirabilis in UTIs is related to its specific pathogenic ability. It has the potentials to colonize the urinary tract by tolerating the local pH and swarm by the aid of fimbriae. P. mirabilis also has the ability to evade host defenses by producing IgA which degrade protease and finally damaging the host tissue with urease and hemolysins [15,16,17]. According to the Center for Disease Control [18], Proteus is responsible for 3% of nosocomial infections in American hospitals.

From this study the significance on the distribution of Proteae isolates related to age was explicit. Recovery of Proteae isolates was mostly from urine of patients aged 0-10 and > 50 years. The young have a less developed immune system and for the elderly a system that warns out with time. Age specific infections rate could also be due to the fact that P. mirabilis mostly infects host compromised by underlying diseases or abnormalities. The elderly patients have increased risk factors predisposing them to infections by Proteae. Risk factors like impaired host defense which occurs in cases of underlying diseases. Though macrophages and polymorphonuclear leukocytes functions are not affected by age, the quality and quantity of antibodies produced by B-cells are jeopardized thus increasing susceptibly to bacterial infections.

CSF and blood are generally sterile in nature, but the presence of any microbe is indicative of infection and polymicrobes implies contamination during laboratory culture procedures. Isolation frequency of Proteae isolates from blood samples of patients according to age revealed that patients aged 0-10 years had the highest rate of infection. Neonates are generally easily infected by various bacteria at birth. This is due to the nature of the vagina at pregnancy which favors replication of bacteria. As such during birth the neonate becomes infected at the level of the birth canal. Ovalle et al. [7] reported M. morganii to be the cause of sepsis in premature infant and a cefotaxime resistant sepsis in a 17 days neonate infected at birth. Other factors could have well been responsible for sepsis in patients age 0-10 years caused by Proteae.

Proteae bacteremia in the elderly (>50) may be associated to operative procedures on the lower urinary tract which requires the use of urinary catheters. Bacteremia may be provoked
isolated from a diabetic foot ulcer. Previous findings [6] in which this organism was M. morganii the others from urine and blood. Recovery of recovered two were from wounds and burns and In this study, from the four M. morganii isolates recovered two were from wounds and burns and the others from urine and blood. Recovery of M. morganii in wounds and burns is consistent with previous findings [6] in which this organism was isolated from a diabetic foot ulcer. M. morganii infections are uncommon but easily observed in HIV/AIDS patients, neonates and the elderly. The significant relationship between Proteae species and the age range group in this study shows that there was a high percentage of P. mirabilis from patients age 0-10 years followed by those >50 years. This is in accord with another report [1]. P. penneri was obtained in urine of patients aged 0-10 years; and it was probably due to contamination of the urinary tract by fecal residues, since P. penneri had always, in the past, been associated with outbreaks of diarrheal infections and kidney stones formation. The high rate of recovery of Proteae from patients in the surgery service and urinary tract infections explains the fact that most patients may have just been cross contaminated. Infection may have resulted from improperly sterilized equipments, contaminated sterile surgery material, infected personnel and environmental factors such as bedding and post-surgery catheters.

In this study, of a total of 1539 samples obtained from out-patients, only 19 (0.01%) Proteae isolates were recovered. This indicates that Proteae species in out-patients may have also resulted from specimen contamination or patients’ contamination on their passage in the consultation room. Such assumption suggests the possibility of cross-infection presumably from contaminated or unsterilized equipments.

Most Proteae isolates obtained in this study where susceptible to imipenem, ceftazidime, chloramphenicol, gentamicin, nalidixic acid, ofloxacin and amikacin. P. mirabilis and Prv. alcalifaciens were readily distinguished by their marked susceptibility to the eleven antimicrobials. Prv stuartii was found to be 100% resistant to gentamicin, tobramycin and chloramphenicol but had marked susceptibility to ciprofloxacin and ceftazidime. Spach et al.[22] explained that there have been decrease in the susceptibility of ciprofloxacin due to its routine usage. Prominent resistance was also observed in Prv rettgeri to tobramycin, nalidixic acid, ciprofloxacin, amoxicillin and chloramphenicol. This observation is in conformity to findings from O’Hara et al. [1]. According to this author, Prv rettgeri resistance to tobramycin and gentamicin could be attributed to excessive usage of these drugs. Biedenbach et al. [23] also studied the susceptibility of Proteae organisms to gentamicin, imipenem, ciprofloxacin, tobramycin and found similar the results with those of this study.

To determine the epidemiological patterns of Proteae infections in the different service areas, Dienes test for colony compatibility was done. Strains involved in cross-infection in the surgery, medicine and out-patients department were detected. Results suggested that Dienes reactions could be due to bacteriocin-like substances. Dienes phenomenon can easily be observed when clinical isolates are labeled with fluorescent proteins, but however can be mediated by UV light or bacteriocins [24]. Dienes compatibility strains are to some extent related. Colony compatible strains observed in this study could have been due to case to case transmission as a result of inadequate nursing techniques in these service areas. Dienes positive reactions could be taken as indication that the strains were different while Dienes negative reactions should not be taken as a reliable test of identification. However Dienes reaction was the criterion used to determine strains involved in nosocomial cross infection in this research.

Lack of full information on patient’s history (sex, duration of hospitalization, medical prescription and clinical syndrome) and poor specification on urine sample collection are some of the limitations of this study. Knowledge on catheterized and non-catheterized urine would have been an essential parameter for prevalence studies.

**Conclusion**

There was variability in the frequency of isolation of Proteae from clinical specimens. In this study, the Proteae isolates were obtained from urine, wounds and burns, blood and CSF. Of the bacterial isolates obtained from specimens in this
research, 18.1% were caused by Proteae species of which P. mirabilis was predominant, followed by P. vulgaris and Prv. alcalifaciens. P. penneri are rarely isolated from clinical specimens but in this research five were isolated. Most Proteae isolates were obtained from urine clinical samples. Epidemiological patterns of Proteae infections in the service areas showed that most isolates were from in-patients with the least from out-patients. High prevalence of Proteae infections was probably due to the extended time of hospitalization and the overcrowding nature of the wards. All Proteeae isolates from this study exhibited resistance to antibiotics commonly used in hospitals. However, they were susceptible to ceftazidime, ofloxacin, nalidixic acid, ciprofloxacin and gentamicin. Prv. stuartii, Prv. rettgeri, P. vulgaris were readily resistant to antibiotics comparatively to P. mirabilis and Prv. alcalifaciens which were easily susceptible to antimicrobials. M. morganii, Prv. stuartii and P. penneri were the Proteae most resistant to antimicrobials.

There was evidence of nosocomial cross-infection especially in the medical and surgical services of the hospital. The control of nosocomial infections caused by Proteae requires constant surveillance on their epidemiological profile and the routine usage of antimicrobials in hospitals.

ACKNOWLEDGEMENTS
The management and staff of the Laquintinie Hospital in Douala, Cameroon are hereby appreciated for permitting and participating in this study.

REFERENCES


