COMPARISON OF ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF SOME BACTERIAL ISOLATES AND PREVALENCE OF ANTIBODIES TO SOME COMMON PATHOGENS FROM HIV-1 INFECTED PATIENTS AND HIV-1 SERONEGATIVE CONTROLS

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DECLARATION

I Simon Andrew Dielly, hereby declare that this dissertation is entirely my own product and that it has never been submitted to any other university for any other degree or diploma.

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DEDICATION

This dissertation is dedicated to

My late Father Rev. Swirthern W. Lwanda

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ABSTRACT

235 newly admitted patients into the medical wards of the Muhimbili Medical Centre in Dar es Salaam, Tanzania were investigated for antibiotic sensitivity patterns of bacterial isolates from the nostrils and urine samples obtained from HIV-1 infected/AIDS patients and HIV-1 seronegative referents. Immunoglobulin levels, serum antibodies against Treponema pallidum, Salmonella spp, staphylolysin, and Group A Streptococcus streptolysin O were determined in the HIV-1 seropositive and HIV-1 seronegative referents. All staphylococcal isolates from the nostrils of HIV-1 infected/AIDS and HIV-1 seronegative referents were sensitive to vancomycin and oxacillin but there was significant difference ($P=0.003$) in the mean inhibition zones of vancomycin in the two groups. There was a significant ($P<0.001$) difference in resistance to chloramphenicol and doxycycline shown by staphylococcal isolates from the nostrils of HIV-1 infected/AIDS patients and HIV-1 seronegative referents. There was also a significant difference ($P<0.001$) in sensitivity pattern to cefuroxime in the two groups investigated. Resistance to antibiotics which are rarely or not used at all in Dar es salaam was not noted in the studied patients. Significant ($P<0.001$) difference in sensitivity to chloramphenicol and gentamicin was noted from the urine isolates from HIV-1 seropositive and HIV-1 seronegative referents. Bacterial isolates from HIV-1 infected/AIDS patients were therefore significantly ($P<0.005$) more resistant to routinely used antibiotics than isolates from HIV-1

seronegative referents.

There was a significant difference (P=0.003) in the prevalence of antibodies (IgM and IgG VDRL and TPHA respectively) against Treponema pallidum in HIV-1 infected patients compared to HIV-1 seronegative referents indicating that HIV-1 infected patients were more likely to have had infection with T. pallidum than their HIV-1 seronegative referents. There was a difference in the levels of antistaphylolysin in the sera of HIV-1 infected/AIDS patients and HIV-1 seronegative controls. The mean ASOT titres in the HIV-1 infected/AIDS and HIV-1 seronegative controls were 288 and 343 Todd units respectively but there was no significant difference (P=0.497). There was also no significant difference (P=0.652) in the prevalence of salmonella antibodies in the HIV-1 infected/AIDS patients and the HIV-1 seronegative referents.

There was a significant difference (P<0.001) in levels of IgG (mean =34g/L), IgA (mean=4.7g/L) and IgM (mean=4.03g/L) in HIV-1 seropositive patients compared to HIV-1 seronegative referents who had IgG levels (mean=25.3g/L), IgA (mean=3.9g/L) and IgM(mean=3.81g/L). Generally levels of Ig in the studied population were higher compared to a Tanzanian healthy adult population which are IgG, IgM, IgA as 18.55g/L, 2.51g/L and 2.51g/L respectively [27].

The present study suggests that HIV-1 infected/AIDS patients are more prone to bacterial infections with common pathogens. The results also suggest that HIV-1 infected/AIDS patients tend to harbour bacterial flora which are multiply resistant to antibiotics.

1. INTRODUCTION

1.1: IMMUNODEFICIENCY PROBLEM.

Patients with defects in host defence mechanisms present an important and increasing problem in medical practice. While severe primary immunodeficiency diseases are rare, patients with secondary or acquired immunodeficiency disorders are commonly encountered, particularly in clinical medicine. It has been known for many years that certain patient groups have depressed host defences against microbial infections. Such patients may be broadly classified into those with primary defects in defence mechanisms and those with secondary or acquired defects. Patients in the latter group have become increasingly important in clinical medicine during the last two decades. Such patients may today be seen by practitioners at any level and collectively they occupy a considerable number of beds in hospitals [1]. But of recent, world-wide concern is directed at the acquired immunodeficiency syndrome (AIDS) pandemic and associated opportunistic infections and malignancies.

1.2: IMMUNODEFICIENT STATES AND ASSOCIATED INFECTIONS.

1.2.1: Neutropenia

The decrease in numbers and functions of several neutrophil parameters have been shown to play a role in the development of infectious diseases. The severity of neutropenia is an important factor which increases the risk of infection in inverse proportion to the neutrophil count in blood

when this falls below 1000 per cubic millilitre (cu. ml). The risk increases steeply below 500 neutrophils per cu. ml, and becomes overwhelming with a neutrophil count below 100 per cu. ml [1]. The duration of severe neutropenia is another important predisposing factor to infection [1].

Infections in neutropenic patients can afflict almost all organs and tissues, but certain localizations predominate [1]. The most important sites of infection in addition to septicaemia are the upper and lower gastrointestinal tract with stomatitis, pharyngitis, oesophagitis and perianal cellulitis; the respiratory tract with pneumonia as the most important infection, and less frequently the urinary tract may also be infected.

The most important microbial pathogens in neutropenic patients are Staphylococcus aureus, Staphylococcus epidermidis, enterobacteria, Pseudomonas aeruginosa, Candida spp and Aspergillus spp [1]. A number of other bacteria may also be encountered as pathogens in these patients including corynebacteria and Bacillus spp, organisms which in most other clinical contexts are usually discarded by microbilogists as contaminants [2].

1.2.2: Cell-mediated (T-cell mediated) immunodeficiency.

Primary T-cell deficiency is rare, but secondary defects are much more frequent, and are seen in a number of clinical situations including many haematological neoplasms, bone marrow and kidney transplants. Bone marrow

and kidney transplant patients suffer from secondary T cell deficiency because of receiving immunosuppressive therapy too [1]. AIDS is a recently recognized and a typical illustration of the consequences of severe T-cell deficiency. Nearly all major groups of microrganisms may cause infectious problems in these patients [1], although the frequently encountered pathogens include viruses, fungi, parasites and intracellular bacteria.

1.2.3: Humoral (B-cell mediated) immunodeficiency.

Severe B-cell deficiency is seen in primary immunodeficiency states such as Bruton's hypogammaglobulinaemia and the many varieties of "late onset hypogammaglobulinaemias", and is also encountered in secondary immunodeficiency disorders such as multiple myeloma, Waldenstrom's macroglobulinaemia, chronic lymphocytic leukaemia and in association with some lymphomas [1]. As the disease progresses in these secondary immunodeficiency states and as more aggressive treatment is instituted, other defence mechanisms other than the B-cell system become compromised with consequences in changing patterns of infections. However, in the patient with predominantly B-cell deficiency, the most typical bacterial infections encountered are with encapsulated, extracellular pyogenic bacteria such as the pneumococcus [1].

1.3: PATHOGENESIS OF HIV INFECTION.

1.3.1: Destruction of T-4 lymphocytes.

The selective tropism of the HIV-1 for T-4 lymphocytes leads to T-4

depletion and consequently lowering of cell mediated immune (CMI) response, giving rise to a variety of opportunistic infections [3,4]. Many, if not all of the clinical features of infection with HIV-1 can be ascribed to profound immunodeficiency which develops in infected individuals. Later, polyclonal activation of B-lymphocytes is manifested by a rise in serum immunoglobulin concentration, affecting IgG, IgA, and IgM. In later stages of the disease Ig concentrations tend to fall [5-10].

1.3.2:Abnormality of B-cell function

A fall in titre of antibodies to HIV-1 core protein p24 is associated with poor prognosis [11]. HIV-1 infected individuals progress towards AIDS despite early activation of a specific immune response [11-13]. It has been noted that spontaneous secretion of Ig was elevated in patients with AIDS. Their lymphocytes however could not be triggered by pokeweed mitogens to produce Ig. In fact, for the patients with AIDS the levels of Ig measured in cultures triggered by pokeweed mitogen were substantially different from those measured in unstimulated cultures. A defect in Ig production induced by pokeweed mitogen can be due to a defect in T-cells, B-cells or both [14]. The peripheral blood of patients with AIDS appears to lack resting B-cells and has increased number of fully differentiated B-cells [14]. These patients have elevated levels of serum IgG and IgA, a finding which has been reported by others and has been interpreted as evidence of intact humoral immune response [15-17]. The failure to diagnose many of the infections seen in this group of patients

serologically may also be related to this B-cell abnormality [14].

Abnormal B-cell function is also a persistent finding in AIDS as well as in other HIV-1 related conditions. A polyclonal B-cell activation resulting in hypergammaglobulinaemia was described early in AIDS and AIDS related conditions [18,19]. HIV-1 seropositives with high IgG levels have a poor prognosis. It is not clear, however, whether this is due to long standing HIV-1 infection or due to aggressive course of the infection. However in one study, the five men who later developed AIDS had on average an IgG level of 1.5g/L higher than the HIV-1 seropositive men who did not develop AIDS [20]. Oligoclonal IgG bands reflect the IgG increase and indicate long standing HIV-1 infection and poor prognosis [20]. In studies of B-lymphocyte function in 12 homosexual male patients with AIDS, and 12 homosexual controls, the patients were found to have elevated numbers of B-cell spontaneously secreting Ig, decreased B-cell proliferative response to T-cell independent B-cell mitogens and qualitatively deficient helper T-cells [14]. The hyperactive spontaneous B-cell responses as well as refractoriness of signals for T-cell independent B-cell activation were highly suggestive of an in vivo polyclonal activation of B-cells and may have been responsible for the manifestations of B-cell hyperactivity, such as hypergammaglobulinaemia, seen in these patients. It was thus concluded that the scope of immune dysfunction in AIDS involves B-cells as well as T-cells [14].

In a single case, the ability of a patient's B-cells to synthesize Ig in vitro was found to be normal [21]. Yet in one study, the number of

circulating B lymphocytes spontaneously secreting antibody in patients with AIDS was found to be approximately 10 times that in a control group [14]. This pattern of hypergammaglobulinaemia in HIV-1 infection is distinct from patterns observed in other infectious diseases. For example in Epstein- Barr virus (EBV) mononucleosis, IgM and IgE isotypes are most strickingly elevated in the first weeks or months after disease onset while IgD is generally the least elevated among the five Ig isotypes. However none of the Ig isotypes are elevated more than three-fold in normal subjects [20]. In bacterial pneumonias in HIV-1 seropositives, Ig are rarely elevated above normal levels [22]. In patients with tuberculosis where IgM levels are often high, IgD is present at normal or subnormal levels [23], and in patients with other bacterial and fungal infections, IgD may be elevated, although no consistent correlation exists between increased IgD and chronic infections [24]. The particular pattern of hypergammaglobulinaemia found in individuals infected with HIV-1 appears to be unique and suggests that the B-cell activation in this disease is independent of EBV infection or reaction or the result of other secondary infections [25].

Serum IgD levels in individuals infected with HIV-1 were studied as a means of monitoring the character and of timing B-cell activation in individuals with infection. Significantly increased levels of IgD were found to be characteristic of homosexual men who were HIV-1 seropositive but asymptomatic or mildly symptomatic [24]. The hyper IgD globulinaemia became progressively more pronounced in patients with increasingly severe

infection and reached its most marked level in patients with AIDS-related complex (ARC). In ARC patients, IgD levels were increased 8.8 - fold above normal which was disproportionately greater than the 2.4 - fold increase in IgG, the 1.8 fold increase in IgA and the 1.6 - fold increase in IgM. IgD levels declined in AIDS patients (although remained elevated compared to controls). It has been suggested that an unusual type of B-cell activation is responsible for the unique pattern of hypergammaglobulinaemia seen in this disease and that the B-cell activation occurs early in the pathogenesis of HIV-1 infection, often before development of symptoms and continues through out the course of infection [25]. A prevalent and often early reflection of this phenomenon is hypergammaglobulinaemia which is present in more than 90% of AIDS patients [11], and is a common abnormality noted in patients with ARC. While IgG and IgA are the most frequently elevated isotypes, increased to 49% and 65% respectively in AIDS patients, elevated IgM levels have been noted in 24% of AIDS patients [12]. Within the IgG isotype, it is the IgG2 and IgG3 subclasses that are preferentially elevated [4].

In Tanzania there are no studies which have been done on immunoglobulin profiles in HIV-1 infected individuals to date. A few studies done in Dar es Salaam, Tanzania reported on mean levels in healthy adults of IgG, IgM, and IgA as 18.55g/L, 2.51g/L and 1.51g/L respectively [26]. Another study reported on IgG, IgM, and IgA mean levels in healthy adults as 18.24g/L, 1.86g/L and 2.12g/L respectively [27].

1.4: LABORATORY DIAGNOSIS OF HIV INFECTION

The HIV was first isolated by F. Barre-Sinoussi and her colleagues at Pasteur Institute in Paris in 1983 [9]. This was followed by development of the initial serological tests which became indispensable scientific tools to define the extent of the HIV-1 infection epidemic [28,29]. The use of such tests in screening blood donors has considerably reduced blood transfusion as a route of HIV-1 transmission [30]. Several test formats are now in use for the detection of anti-HIV-1 antibody, including enzyme immunoassays [31], Western blot (immunoblot) [32,33], indirect immunofluorescence assay [34] and radioimmunoprecipitation [34]. Recently, more rapid, simple and inexpensive tests for detection of HIV antibody have been developed for use in poorly equipped laboratories [35].

1.5 NORMAL HUMAN BACTERIAL FLORA

The skin and mucous membranes always harbour a variety of microorganisms which can be arranged into two groups: (a) The resident flora which consist of relatively fixed types of microorganisms which are regularly found in a given area at a given age. If disturbed, it promptly re-establishes itself. (b) The transient flora consists of nonpathogenic or potentiallly pathogenic microorganisms which inhabit the skin or mucous membranes for hours, days, or weeks. The transient flora is derived from the environment, does not produce disease, and does not establish itself permanently on the surface [36]. Price (1938) has shown that the amount of surface bacterial flora is influenced by sex in some parts of the body

[37]. In one study it was shown that the nose carried the most bacteria, but there was no significant difference between the counts in the nose and axilla [38,39]. Members of transient flora are generally of little significance as long as the normal resident flora remains intact. However, if the resident flora is disturbed, transient microorganisms may proliferate and produce disease [40].

Members of normal flora may produce disease under certain circumstances. These organisms are adapted to the non invasive mode of life defined by the limitations of the environment. For example the human intestinal normal flora maintains a delicate balance between its population members. There are over 400 species [38], and one gram of faeces may harbour 10^{12} bacteria. If forcefully removed from the restrictions of that environment and introduced into the bloodstream or into other tissues these organisms may become pathogenic. They may also produce disease if introduced into foreign locations in large numbers and if predisposing factors are present. For these reasons, members of the resident flora that are found in disease are sometimes referred to as 'opportunists' [40].

1.6: BACTERIAL INFECTIONS ASSOCIATED WITH HIV-1 INFECTION AND AIDS.

Patients with AIDS have a major deficiency of cellular mediated immunity, therefore they are frequently infected with several microorganisms including viruses, fungi, parasites and intracellular bacteria which in normal individuals are usually checked by intact defence mechanisms.

The commonest infections seen in AIDS patients in temperate countries include Pneumocystis carinii infection which often develops despite the presence of specific antibodies to the organism. Although the cellular response to the organism is regarded as more important for protection, in children with cellullar immunodeficiency specific IgG antibodies to P. carinii have been reported to protect against P. carinii [41,42].

The frequency of nine reactivating or opportunistic infections and Kaposi's sarcoma among patients with AIDS was reviewed in one study where Mycobacterium avium-complex was found to be most significantly common [43]. Organisms such as Mycobacterium tuberculosis and Mycobacterium avium complex are reported to be present in as many as 50% of AIDS patients [44]. In one study, tuberculosis and toxoplasmosis each was significantly common in Africa also in Latin America ($P<0.001$) compared to Europe and USA, while disseminated Mycobacterium avium-intracellulare infections were relatively much rarely present in Africa as well as in Latin America compared to Europe and USA [45]. The data in one study suggested that the pattern of infections in AIDS patients could give clues about transmission of HIV-1 and that there may be a hierachy of reactivation of latent infections in which populations with exposure to multiple agents manifest these preferentially to P. carinii [43]. Infections known to be indicative of such immunodeficiency may be grouped into two categories, reactivations and opportunists. Infections that remain latent in normal hosts but may reactivate during cell mediated immunosuppression include P. carinii

pneumonia, toxoplasmosis, tuberculosis and herpes virus infections. Opportunistic agents are those to which many persons may be exposed but only cause illness in the debilitated hosts.

Opportunistic infections that are at least moderately specific for depressed cell-mediated immunity (CMI) include disseminated salmonellosis and fulminating cryptosporidiosis and those due to Cryptococcus neoformans and disseminated infections with Mycobacterium avium-intracellulare [43]. Members of Mycobacterium avium complex(MAC) are the second most frequently isolated group of mycobacterium in the US Department of Health and Human Services Laboratory [45]. Recent studies from USA suggest that 40-50% of all patients with AIDS are infected with MAC. Genetic analysis revealed that 98% of patients with AIDS harboured only M. avium regardless of anatomic or geographic source of isolate; while approximately 40% of MAC isolates from patients without AIDS were M. intracellulare [46]. Recently in USA it has been noted that the clinical features of tuberculosis developing in the HIV-1 seropositive group tend to be those typically associated with tuberculosis in immunocompetent persons rather than, as described in most previous reports [47], having atypical manifestation. With the increased incidence and knowledge of this syndrome, it has become evident that certain bacterial infections may also occur more frequently or with increased severity during AIDS [47-49]. In Tanzania 25% and 30% of females and males respectively admitted to hospital with tuberculosis in 1990 were HIV-1 seropositive (personnal communication with Dr. Mtoni). No studies have been done in Tanzania on MAC todate but there are cases

of atypical mycobacterium which have been reported so far in patients infected with the HIV-1 in the country.

Infection with nontyphoid species of Salmonella were first described in patients with AIDS in 1983 and since then there have been many reports [50-52]. These infections may occur in persons infected with the HIV before other serious opportunistic infections develop. Since an infection with Salmonella may respond to therapy more predictably than many other infections occuring in persons with AIDS, its accurate diagnosis is particularly important. In the same study, salmonellae were found to be the second commonest cause of bacteraemia after Staphylococcus aureus [53-57]. Salmonella infections have been described as one of the complications occurring in patients with AIDS. However unusual manifestations of recurrent salmonella infection, such as brain, kidney and soft tissue abscesses, have been described rarely [56-59]. In one study, a 36 year old homosexual man seropositive for HIV had a tender, flactuant, subcutaneous mass (5x2cm) in the right subclavian region of the thorax. A needle aspirate from the abscess yielded a purulent fluid, and culture of this fluid grew Salmonella typhimurium [60].

Historically, Staphylococcus aureus has been regarded as an opportunistic pathogen, while the coagulase negative staphylococci (including some strains erroneously identified as micrococci) have been regarded generally as non pathogens. This distinction has been very useful for the diagnosis in the clinical laboratory, but accumulating evidence strongly suggests that coagulase-negative staphylococcus can cause a

variety of pyogenic infections such as pyomyositis [61,62]. Staphylococci have received a great deal of attention because they cause a large number of infections in humans and animals. They are also a major group of bacteria inhabiting the skin. While most research has been carried out on the more pathogenic Staphylococcus aureus, the pathogenesis of the whole genus of staphylococcus is still unclear. In addition, the relationship between staphylococci and the compromised host has only been recognized recently [63]. Nasal septal abscess (NSA) defined as a collection of pus between cartilaginous septum and the overlying mucoperichondrium, has been described in a patient with AIDS [63,64]. Fischl and Dickinson recently reported a series of 42 cases of sinusitis occuring in AIDS patients, with S. aureus being the third most frequent bacterium isolated (after Escherichia coli and Haemophilus influenzae) [64]. One published study noted that infection due to staphylococci may be unusually common in AIDS suggesting that clinicians need to be mindful of that organism in such patients [55].

Though the epidemiology of the HIV-1 epidemic in Africa has been well described [65], the clinical problems associated with HIV-1 induced immunosuppression has received less attention. A few studies which have been done in the industrialised world have shown that several non-opportunistic bacterial pathogens are responsible for HIV-1 related problems in adults [66-68]. Community-acquired pneumonia, usually due to Streptococcus pneumoniae or Haemophilus influenzae, is common both in HIV-1 infected individuals and in AIDS. High incidence rates of pneumococcal

bacteraemia have been reported in AIDS patients in United Kingdom (UK) [69-71]. Pneumococcal pneumonia is a major cause of hospital admission in Africa, particularly among young men who have recently moved to the cities [72]. Despite the importance of these acute bacterial infections and their clear association with HIV-1 in the industrialised world this relation has only been reported in one study in Kenya in Africa. In the study it was reported that acute bacterial infections are causing at least a quarter of HIV-1 related medical admissions in the largest hospital in East Africa [73]. Studies done in the USA and Europe suggest that Pneumocystis carinii is the commonest cause of pneumonia in patients with AIDS [74], with the exception of a series of patients with Streptococcus pneumoniae infection [75]. However reports of pneumonia caused by classic pyogenic bacteria have been rare in patients with AIDS in Europe [75].

1.7: MECHANISMS OF DEVELOPMENT OF RESISTANCE BY BACTERIA TO ANTIMICROBIAL AGENTS.

1.7.1: ANTIBACTERIAL CHEMOTHERAPY

In the past two decades, there has been unprecedented activity in the discovery and development of new antimicrobial agents. One needs only to reflect on the long list of new penicillins, cephalosporins and quinolones that were not available in 1965 to form the opinion that such activity was the result of unrelated research and discovery in many different areas [76]. Technical advances in penicillin chemistry, for example, helped to push the subsequent rapid development of cephalosporins and other beta-

lactams. Advances in the understanding of bacterial physiology led to development of structural activity relationship (SAR) used to direct the chemical modification of antibiotics to improve their antibacterial activity. This approach also led to the discovery of new antibiotics that were active against bacteria that had acquired resistance to older agents.

Upon the introduction of penicillins in the early 1940s, over 80% of Staphylococcus aureus isolates were susceptible to < 0.1 microgram of penicillin G per millilitre. However, penicillin resistant staphylococci appeared within 3 years of the wide spread use of this antimicrobial agent, and by 1948 up to 50% of hospital strains were resistant to it [75-77]. Despite the occasional isolation of resistant strains, pulmonary isolates of Streptococcus pneumoniae remain susceptible to penicillin G and synergy is still commonly demonstrated between penicillin G and aminoglycosides against Enterococcus faecalis [78,79].

Semisynthesis around the 6 APA of penicillin G produces methicillin, oxacillin, nafcillin and cloxacillins, and the resistance of these compounds to inactivation by penicillinase earned them a prominent position in treatment of staphylococcal infections. However by 1961 the first methicillin resistant strain of Staphylococcus aureus was isolated [80], and became a source of nosocomial infections in Europe in the 1960s and 1970s. This type of resistance was rare in the USA until late 1970s [80,81]. Methicillin resistant staphylcocci have steadily increased in frequency, remaining a major nosocomial pathogen primarily at large tertiary care hospitals and nursing homes with a large percentage of

chronically ill patients [82,83]. Due to its activity against staphylococci, vancomycin has become the agent of choice for methicillin resistant isolates [84].

Large quantities of antibacterial agents are prescribed for symptoms of urinary tract infection in general practice without bacteriological confirmation or before laboratory report is received. Also the distribution of organisms causing infections in domicilliary and in hospital practice is different, but few large studies have been undertaken to define this difference [85,86]. Since antibiotics are needed in clinical practice to help patients overcome their infections, we must select the most suitable agent which has least side effects in the particular clinical situation.

Emergence of resistant bacterial strains implies an inevitable threat to antibiotic therapy with the affected antibiotic, be it in outpatients or inpatients. Emergence of resistant bacteria is a consequence of the use of antibiotics in a particular environment. One main reason for this problem is new categories of patients. The immunosuppressed patients with malignant diseases, granulocytopenia, following organ transplantation, severely injured patients, burned patients, patients who have undergone major surgery, those with indwelling catheters or other foreign bodies, alcoholics and AIDS patients fall under this category. These seriously ill patients share two characteristics. They require antibiotic therapy for infectious complications and some have to be treated in intensive care units. These

circumstances will create problems particularly for broad spectrum-antibiotics in closed hospital settings and often for too prolonged periods of therapy. It is our responsibility as doctors to take part in the control measures of antibiotic use both within and outside hospitals, may be by giving mild advice to clinicians or by using more drastic methods [86]. For example giving information to the clinician regarding the most prevalent pathogens from patients under their care and their sensitivity patterns to relevant antibiotics are of importance for selection of effective antibiotic regimens.

A study done in Sweden, for instance, on susceptibility testing on faecal flora indicated that a combination of trimethoprim/sulfonamide was not a better choice than ciprofloxacin or norfloxacin for oral antibiotic prophylaxis in abdominal surgery [87]. It also showed that isoxazolypenicillin or vancomycin was now necessary to cover the gram positive bacteria causing septicaemia in patients with haematological disease [87]. In Muhimbili Medical Centre, Dar es salaam, Tanzania in 1979/80 for instance 1.25 tonnes of tetracycline were used as opposed to only 10 kg of nitrofurantoin. Indeed resistance was only 10-30% with all antimirobials whose annual consumption was less than 10 kg; while 80% of the isolates were resistant to tetracycline [88]. In the same study no isolate were resistant to gentamicin; but there was tendency for 1979 isolates to become more resistant to the drugs tested. Another study done in Dar es salaam showed that about 90% of S. aureus isolates were found resistant to penicillin in the years 1978, 1979 and 1982 [89].

It should be noted that it often takes several years to accumulate enough numbers of clinical isolates to establish the local invitro perfomances of different antibiotics and when obtained, the data may no longer represent the current situation. Therefore up to date information on antimicrobial susceptibility patterns of isolates is constantly needed.

1.7.2:Mechanisms of Development of Bacterial resistance to Antibiotics

It has been established that microorganisms employ different mechanisms to overcome the effects of antibiotics and other antimicrobial chemotherapeutic agents. This is effected by genetic make up (chromosomal) and by transfer/acquisition of plasmids. In gram-negative bacteria, genes governing resistance to such agents as neomycin, kanamycin, streptomycin, chloramphenicol, tetracycline, and sulphonamides are found on one or more plasmids in various combinations. In gram positive staphylococci, genes governing resistance to such agents as penicillin, erythromycin and heavy metals (eg mercury) are also found on plasmids [90]. Most plasmid-governed resistance is mediated by enzymatic inactivation of the drug (eg acetylation or phosphorylation), while chromosome-mediated resistance usually reflects a lowered affinity of the target molecule for the drug. The target of chloramphenicol activity is bacterial ribosome but its effect is resisted through a plasmid mediated mechanism by production of acetyltransferase which detoxifies (acetylates the OH-group) of the drug. Tetracycline also works in the same way as chloramphenicol but resistance to it is a result of a membrane protein causing active drug efflux which

is plasmid mediated. Resistance to erythromycin is a result of chromosomal mutation causing lowered affinity of ribosomal protein also through a plasmid product, methylase, causing methylation of 23S ribosomal RNA. Ribosome is a target of aminoglycosides and resistance to these is through chromosomal mutation resulting in lowered affinity of ribosomal protein 16S RNA methylation also by a plasmid product, O-N-acetyltransferase which interferes with binding to the target. Resistance to chemotherapeutic agents which act by interfering with cell wall synthesis (eg beta-lactams [penicillin, cephalosporins]) is mediated through chromosomal mutation and plasmid mediated beta lactamase production. Sulphonamide resistance is through chromosomal mutation where there is lowered affinity of dihydropteroate synthetase, a substitute enzyme. Trimethoprim resistance is chromosomally mediated by production of thymine auxotrophy (spares folate) and a plasmid product, drug resistant dihydrofolate reductase a substitute enzyme [90].

2. RATIONALE OF STUDY

From the literature review, it is apparent that very little work has been done on immunoglobulin profile of healthy Tanzanians [26,27]. There is no study which has been done on immunoglobulin levels in HIV-1 infected patients in Tanzania. Also there are no studies which have been done on prevalence of antibodies to different normal pathogens in HIV-1 infected patients and compare them to HIV-1 seronegative referents in Tanzania.

Two studies have been carried out in Dar es Salaam, Tanzania on susceptibility patterns of Gram positive cocci and Gram negative rods isolated from urinary tract in 1980's [88,89]. No follow up studies have been done since then.

HIV-1 infected patients are frequently infected by normal bacterial pathogens [73], which may have been exposed to substandard doses of antimicrobial agents. Due to this they may with time, develop high level resistance to a number of antimicrobial agents thus making treatment of subsequent infections with these organism difficult. Studies for determination of susceptility patterns of isolates from patients are very much in keeping with the rising use of antibiotics in Tanzania.

It is interesting to study levels of antibodies against different pathogens in HIV-1 infected individuals because the patients are prone to frequent infections.

3. OBJECTIVES OF THE STUDY

BROAD OBJECTIVES

3.1 To compare the antimicrobial susceptibility patterns of some bacterial isolates from HIV-1 infected/AIDS patients with some bacterial isolates from sex and age matched HIV-1 antibody negative referents.

3.2 To determine the prevalence of antibodies to some common pathogens in HIV-1 infected patients and in sex and age matched HIV-1 antibody negative referents.

3.3 SPECIFIC OBJECTIVES

3.3.1 To determine the prevalence of some bacterial species in the nose and urinary tracts of patients infected with HIV-1/AIDS.

3.3.2 To determine the prevalence of some bacterial species in the nose and urinary tracts of HIV-1 seronegative sex and age matched referents.

3.3.3 To compare the prevalence of bacterial species from the nose and urinary tracts of HIV-1 infected/AIDS patients and HIV-1 seronegative referents.

3.3.4 To determine the antimicrobial sensitivity patterns of bacterial isolates from HIV-1 infected/AIDS patients and compare

them with the sensitivity patterns of isolates from HIV-1 antibody negative referents.

3.3.5 To determine the prevalence of antibodies to Salmonella typhi (O9), Salmonella paratyphi B (O4), Treponema pallidum, staphylolysin and to streptolysin- O of Group A streptococcus in HIV-1 infected/AIDS patients and HIV-1 antibody negative referents.

3.3.6 To determine the levels in serum of IgG, IgM and IgA in HIV-1 infected and HIV-1 seronegative referents.

4. PATIENTS MATERIALS AND METHODS

4.1: PATIENTS

This was a cross sectional study and was carried out from 1st June to 20th August 1989.

The patients were recruited from all female and male medical wards (1,3,4,5 and 6) in Muhimbili Medical Centre, Dar es Salaam, Tanzania. The data was collected from all newly admitted patients in the admitting medical wards in the hospital.

The study population consisted of 235 patients. Patients and referents gave informed consent to participate in the study and all identifying information was destroyed before antibody testing to the HIV-1 was done in order to ensure anonymity. HIV-1 antibodies screening by ELISA method was done to all patients in the study.

Information concerning presenting diagnosis and previous medical treatment was obtained from the referral letter and recorded in the patient's questionnaire.

4.2: MATERIALS

Specimens were collected from all recruited patients. These included nasal swabs, early morning mid-stream urine and serum samples. A sterile bottle containing sterile 1% peptone water was used for moistening the dry swab for taking nasal specimens and for transporting the specimen for each patient. Each nostril was swabbed in a standardised circular manner thrice from each patient by the author.

Early morning mid-stream urine specimens were collected in empty sterile universal containers after each patient had been instructed by the author on the previous day and specimens were refrigerated if delay of more than one hour prior to processing in the laboratory was anticipated.

About 5-10 ml of blood was collected from each patient in empty sterile containers and allowed to clot. Centrifugation was done to separate serum which was then aliquoted into 2.5 ml Nunc tubes in triplicate and stored at -20^{0}C without preservatives.

The specimens were processed in both the Department of Microbiology and Immunology at the Muhimbili Medical Centre Dar es Salaam, Tanzania and at the Department of Microbilogy and Immunology, University of Bergen, Bergen, Norway after being transported by air in cold boxes from Dar es salaam. In Dar es Salaam the bacteria isolated from the nostrils and urines of the HIV-1 seropositive and HIV-1 seronegative referents were identified and preserved. Screening for anti HIV-1 antibodies in all the sera was also done in Dar es Salaam.

Bacterial isolations.

For isolation of skin flora, blood agar (BA) and Mackonkey agar (MCA) were used. The culture media used for isolation of bacteria from urine samples included BA and MCA. Identification of all organisms was based on conventional methods described by Cowan and Steel (1970) [91]. For antibiotic susceptibility tests, PDM Antibiotic Sensitivity Medium II (AB BIODISK pyramidvagen 7, S-171 Solna Sweden) was used. Sterile normal saline was used to emulsify isolates picked up by touching with a loop

five colonies of pure culture of each isolate before flooding on PDM plates. Penicillinase medium was used for detection of beta-lactamase production by staphylococci and rabbit plasma was used for detection of coagulase production by staphylococci. Antibiotic tablets were obtained from Denmark (Rosco Diagnostica) [92].

For detection of beta lactamase production, the following medium was used:

Bact agar Difco	1.5 gm
0.2% Phenol red	5.0 gm
Distilled water	100.0 ml
Penicillin G	0.3 gm/100ml
pH 8.5-9.0	

In Bergen isolates were preserved using
Greaves's solution (locally prepared) whose contents are:

Bovine serum albumin	50 gm
Sodium glutamate	50 gm
glycerol	100 gm
Distelled water	1000 ml

4.3: METHODS

The following tests were done in Bergen: anti HIV-1 antibodies screening, immunoblot for anti HIV-1antibodies, serum Ig profiles, screening for antibodies to Treponema pallidum, salmonella, Group A streptococcus and

staphylolysin. Bacterial sensitivity patterns of the isolates from the nose and urinary tract were also determined in Bergen.

4.3.1: Isolates from the nose.

Nasal swabs were inoculated onto BA, MSA and MCA. After overnight incubation at 37^{0}C bacteria were identified as Staphyloccus species when they were gram positive cocci in clusters and were catalase positive. The staphylococcal isolates were preserved in nutrient agar (NA) slopes in Dar es Salaam and later in Greaves solution in Bergen. The isolates were stored at -70^{0}C in Bergen until needed. When needed the isolates were removed from -70^{0}C and left at room temperature to thaw. Subculture was done on BA and only pure cultures were subjected to the following tests: A tube coagulase test in which the isolate was emulsified in rabbit plasma and later incubated at 37^{0}C over night. A positive coagulase test was interpreted when there was a coagulum which could not be broken even after vigorous strocking of the tube with a finger and the coagulum could not be poured after turning the tube upside down.

Beta-lactamase production was detected by acidometric method. Acidometric agar was prepared thus : to 100 ml of distilled water, 1.5 gm of agar and 0.2 ml of 0.5% phenol red solution were added and boiled until the agar disolved. Using 1 N NaOH, the pH was adjusted between 8.5 and 9.0, confirming with pH paper. Then 20 ml portions were dispensed into test tubes and kept at 2 to 8^{0}c (stable for at least 6 months) [93,94]. When needed, a portion of medium was melted and cooled to 50 to 55^{0}c. Penicillin G powder was then added to yield a concentration of 5,000 U/ml.

The tube was mixed well and the pH was rechecked and adjusted if necssary (pH 8.5 to 9.0). The solution was poured into a petri dish and allowed to solidify. The plate was divided into six portions. A clump of colonies was placed in a discrete spot on the plate, as were positive and negative controls (pencillinase producing and non penicillinase producing Staphylococcus aureus). The plate were incubated at 35^0c overnight. Beta-lactamase producing Staphylococcus sp. broke down penicillin G to penicilloic acid which changed the colour of the medium from pink to yellow [94].

For sensitivity tests to antibiotics, a loopful of young culture of staphylococcus on a plate was emulsified in 1 ml sterile normal saline. The 1 ml culture suspension was then flooded onto PDM plates and any excess fluid was pippeted off using a sterile disposable pippete. The susceptibility test plates were left to dry at room temperature. This was used to test sensitivity to oxacillin. From the 1 ml suspension of culture a loopful of the suspension was transfered to 5ml of normal saline in a sterile test tube and mixed throughly, and then flooded onto PDM media and treated as above. This was used to test antibiotics other than oxacillin. An antibiotic disc dispenser supplied by neo-sensitabs (Rosco Diagnostica, Taastrup-Denmark) and antibiotic discs from the same manufacturer were used. To control precision and accuracy of the test procedure, stock culture of Staphylococcus aureus (ATCC 25923) was included as control. The plates were incubated at 37^0C overnight. Sensitivity testing was performed for the following antibiotics: cotrimoxazole, cephalothin, erythromycin,

fucidin, clindamycin, vancomycin, doxcycline, chloramphenicol, netilmicin, cefuroxamine and gentamicin. The sensitivity tests were interpreted according to manufacturer's instructions by measuring the diameter of zones of inhibition in mm around the antibiotic discs using a vernier calliper with sliding scale and graded. The grades were 1 (highly sensitive), 2 (moderately sensitive), 3 (slightly sensitive) and 4 (resistant) [92].

INTERPRETATION OF ZONES OF INHIBITION AND TABLET CONTENTS

	Zone of inhibition in mm and grades			
Antibiotics discs strength	1	2	3	4
Oxacillin 5 microgram	> 20	–	–	< 20
Cefuroxime 66 ,,	> 28	27–20	19–14	< 14
Cephalothin 60 ,,	> 28	27–20	19–14	< 14
Erythromycin 78 ,,	> 28	27–23	–	< 23
Gentamicin 40 ,,	> 26	25–20	–	< 20
Netilmicin 40 ,,	> 26	25–20	–	< 20
Chloramphenicol 60 ,,	> 32	31–26	–	< 26
Doxycycline 80 ,,	> 28	27–23	–	< 23
Vancomycin 70 ,,	> 18	17–15	–	< 15
Fucidin 400 ,,	> 32	–	–	< 32
Clindamycin 25 ,,	> 26	25–18	–	< 18
Cotrimoxazole (240+5.2) ,,	> 28	–	–	< 28
Nitrofurantoin 260 microgr	> 24	–	–	< 24

4.3.2: Gram negative bacterial isolates from urine.

All urine specimens seen after arrival at the laboratory were mixed properly after which 1 microlitre loopful of each specimen was inoculated onto BA and MCA. E. coli (ATCC 25922) was used as control for antibiotic sensitivities on gram negative urine isolates. After overnight incubation at 37^0C organisms growing in significant numbers as defined by the following criteria were identified: (organism growing in pure culture with colony counts of more than 10^5 colony forming units (CFU) per ml). Urine exhibiting mixed growth were not dealt with any further. Identification of each significant pure culture growth was done according to previously described methods [90]. The isolates were later preserved as described above. When needed, the isolates were retrieved from the -70^0C freezer and allowed to thaw at room temperature. The isolates were then subcultured onto BA and incubated at 37^0C overnight. Testing for antibiotic sensitivity pattern and interpretation was done as described for the nasal bacterial isolates.

4.3.3: Quantitation of IgG,IgM and IgA.

This was done using a laser nephelometer (Behringwerke AG) using the principle of immunoprecipitation for antibody assay. The serum samples were removed from the -20^0C freezer and allowed to thaw at room temprature prior to assaying. The machine was first calibrated using standard antisera for human IgG,IgM and IgA. Reference protein, which was recommended by the manufacturer was used as control. Reference curves were drawn for each standard. The buffer used was a solution of hydrophilic

polymers in phosphate buffer solution with sodium azide. The serum samples were run in the nephelometer and the intensity of the precipitate formed was read by photoelectric intensity which recorded the amount of precipitate in grams per litre.

4.3.4: Quantitation of Antistreptolysin O titres (ASOT)

This was done using a laser nephelometer (Behringwerke AG) and the procedure was as described above.

4.3.5: Detection of HIV-1 antibodies

The enzyme linked immunosorbent assay (ELISA) was used for screening of HIV-1 antibodies from patients. The test was done according to the manufacturer's instructions (Organon Teknika). The sample was diluted 1:34 by adding 1,000 microlitres of sample diluent to 30 microlitres of the specimen. Then 100 microlitres of each diluted test sample were put into wells of a microtitre plate coated with HIV-1 antigen. After the addition of the samples in the wells, the wells were covered with a new plate sealer and incubated at 37^0C for 30 minutes, after which the wells were washed four times using a washing machine. Meanwhile conjugate was prepared by dissolving one vial of freeze-dried conjugate (enough for 4 rows of 12 wells each) in 5.5 ml of sample diluent. Then 100 microlitres of conjugate solution were pipetted in each well. The plate was then covered with new plate sealer and incubated at 37^0C for 30 minutes, after which the wells were washed four times using a washing machine. 5 optic

density (OPD) tablets were dissolved in 12.5 ml of distilled water and allowed to dissolve completely for 15 minutes in a dark place. Then 500 microltres of urea peroxide were added to OPD solution to make substrate solution. The solution was almost colourless prior to use. Then 100 microlitres of substrate solution were pipetted into each well, after which the plate was incubated at 25^{0}C in the dark for 30 minutes. The reaction was stopped by adding 100 microlitre of 2mol/L (4N) sulphuric acid in each well. The readings of the results of the 235 sera which were processed on the same day were done using the photometre and also visually and the two readings were compared. The filter used was the 492 nm filter. The readings of the controls were as shown below:

Positive control 1. 0.255

Positive control 2. + 0.362

0.617 divide by 2 = 0.309

Negative control 1. 0.092

Negative control 2. + 0.093

0.185 divide by 2 = 0.093

Cut off 0.402 divide by 2 = 0.201

Any reading below the cut off was regarded as negative and those above the cut off point were taken as positive reaction and were subjected to the Western immuno-blot.

4.3.6: Western immunoblot for HIV-1 antibodies

The procedure was done according to the manufacturer's instructions (Biotech Research Laboratories, Inc. 1600 E. Gude Drive Rockville,

Maryland 20850, USA. U.S. License no. 1035). Briefly the following was done: All reagents were brought to room temperature, then 2 ml of diluted wash buffer were added to each well and then carefully, using a plastic pair of forceps, nitrocellulose strips were removed from their container and placed in numbered wells containing wash buffer. The strips were incubated for 30 minutes at room temperature and then the buffer was removed by aspiration. After that 2 ml of working buffer were added then the tray was put on rotary platform for 10 min at room temerature. Later 20 microlitres of undiluted serum was added in the assigned strip after which the tray was covered and incubated on a rotary platform overnight at room temperature. The mixture in the wells was aspirated and each strip was rinsed once in wash buffer and rewashed once more with 2 ml of diluted wash buffer for 5 min. Then 2 ml of working conjugate solution 1 were added into each well and incubated for 60 min at room temperature on the rotatory platform after which, conjugate was aspirated from all wells and each strip was washed three times using 2 ml of diluted wash buffer for 5 min. 2 ml of working conjugate solution 2 were added in each well and incubated as above and later aspirated and the strips washed thrice. 2 ml of working substrate solution were added in each well and incubated for 15 min on the rotary platform, after which the strips were rinsed with distilled water to stop the reaction. Interpretation of the results was done according to the W.H.O instructions [95].

4.3.7: Tests for Antibodies to Treponema pallidum

The test for detection of antibodies to cardiolipin was done according to the instructions from the manufacturer of Venereal Disease Research Laboratory(VDRL) test kit(Behringwerke AG,Murburg,W. Germany). Briefly the following was done: Samples were inactivated at 56^0C for 30 min prior to testing. 50 microlitres of test serum samples were put in microtitre plate and diluted serially (the final serum dilutions were from 1:8 to 256), then 25 microlitres of cardiolipin antigen were added in all the wells. The plate was subjected to micromixer for 5 min and then centrifuged for 10 minutes. Evaluation was done immediately after rotation under a microscope at magnification of x 100. Uniform turbidity of the suspension with no flocculation or lumps was interpreted as non reactive. Clearly defined lumps in a clear sorrounding was interpreted as reactive. The sera which tested positive by the screening test were tested again by the Treponema pallidum Haemagglutination (TPHA) test (FUJIREBIO INC 6-7,Shimoochiai 4-chome, Shinjuku-ku, Tokyo, 161,Japan) according to the manufacturer's instructions. The following was done: 20 microlitres of patients serum were added to 380 microlitres of absorbing diluent to make a 1:20 dilution. The mixture was left at room tmperature for 30 minutes. Meanwhile sensitized sheep red blood cells (SSB) was prepared by adding 100 microlitres of SSB to 550 microlitre of diluent and non sensitized blood (NSB) by adding 100 microlitres of NSB to 550 microlitre of absorbing diluent. Using microtitre technique 25 microlitre of patients serum were put in a well and then 25 microlitres of complement buffer

were added in other wells in the same row, and serial dilution of serum was done. The final serum dilutions were from 1:80 to 1:2560. Later 75 microlitres of SSB were added in all the wells. Positive and negative controls were subjected to similar procedure. The plate was shaken by hand for 5 min and then incubated overnight at room temperature without being disturbed. Interpretation, the presence of a smooth mat of cells covering the entire bottom of the well and the edges was regarded as reactive. The presence of a definite compact button in the centre was interpreted as non reactive.

4.3.8: Screening for antibodies against somatic antigen of S.typhi(O9), and S.paratyphi B (O4).

The test was done using antigens prepared locally in Oslo (STATENS INSTITUTT FOR FOLKEHELSE OSLO NORWAY). Briefly each of the locally made antigens was dyed using 0.5% safranin in normal saline. The final concentration of antigen was 0.005%. The test serum was diluted 1:4 after inactivation at 56^{0}C for 30min. Microtitre plates were used in this test. Each serum was serially diluted prior to the addition of the antigen. The final serum dilution were from 1:8 to 1:256. Control serum was obtained from the Institute of Public Health, Oslo.

4.3.9: Screening for antibodies against staphyloysin

It was done according to the manufacturer's instructions (Behringwerke). Briefly 100 microliters of patient's serum were added to 900 microliters of antistaphylolysin buffer from the manufacturer (inactivated at 56^{0}C for 30 min) to make a 1:10 dilution. Then in a microtitre plate 50 microliters of inativated patient's serum were put in the first well and 25 microliters of buffer in 8 wells and titration was done (the final serum dilutions were from 1:2 to 1:16) and then 25 microliters of lysin were added to all the wells and incubated at 37 degrees centigrade for 30 min after which 50 microlitres of 2% freshly prepared rabbit serum were added, incubated for 1 hr and then centrifuged. Titres of antibodies against staphylolysin were determined quantitatively by the reciprocals of serum dilution of lysis inhibition.

4.3.10: STATISTICAL ANALYSIS.

The results were analysed by BMDP-ONE SAMPLE AND TWO SAMPLET-TEST (Copyright 1977, 1979, 1981, 1982, 1983, 1985, 1987, 1988 BMDP Statistical soft ware, Inc. California, USA).Significance was estimated at 95% confidence intervals ($P<0.005$)

5. RESULTS

The study group comprised of 235 patients. Of these 139 (59%) were males and 96 (41%) were females. Twenty three (24.0%) of the female patients were HIV-1 seropositive while 50 (36.0%) males were HIV-1 seropositive. The prevalence of HIV-1 infection irrespective of sex was therefore 31.1%.

Age distribution of the studied patients is shown in Table 1. The age ranged from 15 to 56 years. Most of the patients were from the age group 41 to 45 years, which had 48 (20.4%) patients. There was no disparity in age distribution between male and female patients. HIV-1 seropositive females were younger by about 5 years compared to male HIV-1 seropositive (Table 2).

Out of the 235 nasal swabs from 235 patients, 146 (62.1%) yielded staphylococci. One hundred and thirty one (90%) of the staphylococcal nasal isolates were beta-lactamase producers and fifty (34%) were coagulase positive staphylococci. There was no significant difference (P = 0.718) in beta-lactamase producing staphylococci isolates from the nostrils of HIV-1 incfected and HIV-1 seronegative referents. Twenty six (37.1%) out of the seventy staphylococcal isolates from the nostrils of HIV-1 seropositive patients were coagulase producers compared to 24 (31.5%) from a total of 76 staphylococcal isolates from nostrils of HIV-1 seronegative referents (P=0.005).

Out of 12 antibiotics tested, 10 (83.3%) were active against the nasal staphylococcal isolates. Nasal staphylococcal isolates from HIV-1 infected patients showed significantly bigger diameters of inhibition zones to chloramphenicol and doxycycline in both sexes ($P=0.002$) and ($P<0.005$) respectively and to cefuroxime in males ($P<0.001$) compared to nasal isolates of staphylococci from seronegative referents (Tables 3,4). There was significantly lower ($P<0.001$) sensitivity to vancomycin in the nasal staphylococcal isolates from HIV-1 seropositive compared to nasal staphylococcal isolates from HIV-1 seronegative referents. Out of 12 antibiotics tested for sensitivity against staphylococci 7 (58.3%) of the antibiotics did not show any significant difference in sensitivity of the isolates from HIV-1 positive patients and HIV-1 seronegative referents. However, there was significantly more resistance to oxacillin shown by the nasal isolates from HIV-1 positive patients compared to those from HIV-1 seronegative referents ($P=0.008$).

Out of 235 urine samples examined 33 (14.0%) yielded a significant bacterial growth. Nine (27.3%) of the isolates were from HIV-1 seropositive patients and 24 (72.7%) were from HIV-1 seronegative controls. The isolates comprised of Escherichia coli from 20 (60.0%), Klebsiella spp from 11 (34.4%), Proteus spp from 1 (3.0%), and Pseudomonas spp from 1 (3.0%) patients. Isolates from urine samples of HIV-1 seropositive patients showed resistance to chloramphenicol and gentamicin (Tables 5), while gram negative bacterial isolates from urines of HIV-1 seronegative patients showed tendency to have isolates having

large zones of inhibition to the 7 antibiotics tested (Table 5). Gram negative isolates from urines of HIV-1 seropositive patients were noted to have less mean diameters of inhibition to doxycycline compared to gram negative isolates from urines of HIV-1 seronegative referents but the difference was not significant (P=0.007) (Table 5).

One hundred and eighty three sera were assayed for immunoglobulin (Ig) levels and antistreptolysin O (AST) titres while fifty two patients had inadequate sera therefore no assay was done on them. All two hundred and thirty five patients were screened for antibodies against HIV-1, Salmonella O4 and O9 and Treponema pallidum and for antistaphylolysin (Table 6, and 7).

Immunoglobulin levels in the studied samples were as follows: IgG maximum level was 59.5g/L, minimum value was 4.04g/L and the mean value was 28.3g/L(with standard deviation [SD] 0.842g/L). IgM levels maximum reading was >8g/L, minimum was 1g/L and the mean value of 4.04g/L (SD 0.191g/l). IgA levels, maximum reading was >10g/L, minimum of < 1g/L and the mean was 4.117g/L(SD 0.151g/L). The levels of IgG and IgA in HIV-1 positive patients was significantly higher than those in HIV-1 seronegative controls (P<0.001). The levels of IgM in HIV-1 seropositive was not significantly higher than in HIV-1 seronegative controls (Tables 8 and 9). However higher levels of IgM were observed in patients who were previously diagnosed as having had tuberculosis.

High titres of antistreptolysin O, were recorded with mean values of 379.6 and 409.6 Todd units in the HIV-1 positive and the HIV-1 seronegative referents respectively. Prevelance of antibodies against Treponema pallidum were significantly commoner in the HIV-1 positive patients compared to HIV-1 seronegative referents ($P<0.001$). There was no difference in the prevalence of antibodies against Salmonella antigens in the HIV-1 positive patients and HIV-1 seronegative referents ($P=0.586$). There was a difference in mean antistaphylolysin titres in the HIV-1 seropositive patients compared to HIV-1 seronegative referents but not statistically significant ($P=0.011$).

Six (26.1%) of the female patients whose presenting diagnosis was tuberculosis were HIV-1 seropositive and 11 (15.1%) were HIV-1 seronegative (Table 10). 4 (17.4%) of the females who were HIV-1 seropositive presented with gastroenteritis compared to 4 (5.5%) of the female HIV-1 seronegative referents who presented with gastroenteritis (Table 10). Table 11 there was no statistical difference in the studied population.

235 patients who were studied for responses to have used antimicrobials during the one month period before being studied gave the following information: tetracycline 80 (34%), chloromphenicol 57 (24.3%) , penicillin 77 (32.8%) and erythromycin 10 (4.3%). Also 120 (74.1%) who were HIV-1 negative had not used antibiotics compared to 7 (9.6%) who were HIV-1 seropositive. Sixty six (90%) of the HIV-1 seropositives had used antibiotics one month prior to admission (Table 12).

TABLE 1. AGE DISTRIBUTION OF PATIENTS STUDIED

Age group in years	Frequency and %		Total
	Females	Males	
15-20	15 (15.6)	17 (12.2)	32 (13.6)
21-25	17 (17.7)	20 (14.4)	37 (15.7)
26-30	17 (17.7)	19 (13.7)	36 (15.3)
31-35	11 (14.5)	25 (18.0)	36 (15.3)
36-40	14 (14.6)	26 (18.7)	40 (17.0)
41-45	18 (18.8)	30 (21.6)	48 (20.4)
46-50	1 (1.1)	1 (0.7)	2 (0.9)
51-55	2 (2.1)	1 (0.7)	3 (1.3)
>56	1 (1.1)	-	1 (0.4)
TOTAL	96 (41)	139 (59)	235 (100)

TABLE 2. HIV-1 ANTIBODY PREVALENCE IN DIFFERENT SEXES AND AGE GROUPS.

Age group in years	HIV-1 STATUS				
	Females		Males		Total
	HIV+ve	HIV-ve	HIV+ve	HIV-ve	
15-20	2(13.3%)	13(86.7%)	3(17.7%)	14(82.3%)	32
21-25	9(52.9%)	8(47.1%)	8(40.0%)	12(60.0%)	37
26-30	5(29.4%)	12(70.6%)	9(47.4%)	10(52.6%)	36
31-35	2(18.2%)	9(81.8%)	11(44.0%)	14(56.0%)	36
36-40	3(21.4%)	11(78.6%)	8(30.8%)	18(69.2%)	40
41-45	2(11.1%)	16(88.9%)	11(36.7%)	19(63.3%)	48
46-50	-	1(100%)	-	1(100%)	2
51-55	-	2(100%)	-	1(100%)	3
>56	-	1(100%)	-	-	1
TOTAL	23(9.8%)	73(31.1%)	50(21.3%)	89(37.8%)	235

TABLE 3. THE MEAN INHIBITION ZONES OF ANTIBIOTICS AGAINST NASAL STAPHYLOCOCCAL ISOLATES FROM FEMALE HIV-1 INFECTED AND HIV-1 SERONEGATIVE REFERENTS

	HIV positive (NO=23)	HIV negative (NO=44)	P-value
Oxa	19.58	21.94	0.0356
Cepha	33.14	35.16	0.0370
Van	20.17	21.73	0.0017
Clin	35.67	37.17	0.1286
Ery	32.47	31.64	0.8014
Fuci	39.29	40.15	0.4055
Sulf/Tri	33.19	33.87	0.4390
Chlor	23.62	28.75	0.0024
Genti	28.58	30.01	0.1117
Netil	30.12	29.90	0.8609
Cefur	33.81	36.45	0.0334
Doxyc	22.07	26.85	0.0058

KEY:

Cepha	=	cephalothin	Vanc	=	vancomycin
Clin	=	clindamycin	Fuci	=	fucidin
Sulf/Tri	=	cotrimoxazole	Chlor	=	chloramphenicol
Genti	=	gentimicin	Netil	=	netilmicin
Doxyc	=	doxycycline	Oxa	=	oxacillin
Ery	=	erythromycin	Cefur	=	cefuroxime

TABLE 4. THE MEAN INHIBITION ZONES OF ANTIBIOTICS AGAINST NASAL STAPHYLOCOCCAL ISOLATES FROM MALE HIV-1 INFECTED AND HIV-1 SERONEGATIVE REFERENTS.

	HIV-seronegative (N=82)	HIV-seropositive (N=44)	P value
Oxa	22.84	20.36	0.0085
Cepha	36.33	34.68	0.0730
Van	21.80	20.83	0.0033
Ery	32.83	33.06	0.8413
Clin	37.89	35.40	0.0096
Fuci	41.35	39.85	0.1689
Sul/Tr	35.63	34.67	0.3715
Chlo	28.02	24.93	0.0010
Gent	30.13	29.27	0.1460
Net	31.05	30.33	0.2520
Cefur	36.94	33.95	0.0011
Doxy	27.02	24.43	0.0324

KEY:

Cepha = cephalothin Vanc = vancomycin Ery = erythromycin

Clin = clindamycin Fuci = fucidin Sulf/Tri = cotrimoxazole

Genti = gentimicin Netil = netilmicin Cefur = cefuroxime

Doxyc = doxycycline Oxa = oxacillin

Chlor = chloramphenicol

TABLE 5.THE MEAN INHIBITION ZONES IN MM OF ANTIBIOTICS AGAINST URINE GRAM-NEGATIVE BACTERIAL ISOLATES FROM HIV-1 INFECTED PATIENTS AND HIV-1 SERONEGATIVE REFERENTS

DRUG	HIV-1 infected (NO=9)	HIV-seronegative (NO=24)	Pvalue
Sul/tri	25.25 SD 9.18	28.15 SD 12.40	0.4930
Chlor	15.50 SD 7.25	24.35 SD 7.25	0.0027
Genti	19.45 SD 6.05	26.27 SD 5.08	0.0044
Neti	23.50 SD 3.49	26.62 SD 5.22	0.1298
Cefur	23.43 SD 2.79	30.84 SD 7.74	0.0139
Doxy	13.08 SD 3.06	20.40 SD 6.85	0.0073
Nitro	25.35 SD 3.33	24.81 SD 5.04	0.7812

KEY:

Sulf/Tri = cotrimoxazole
Chlor = chloramphenicol
Netil = netililmicin
Cefur = cefuroxime
Oxa = oxacillin
Genti = gentimicin
Doxy = doxycycline

TABLE 6. ANTIBODY TITRES (RECIPROCALS OF SERUM DILUTIONS) TO DIFFERENT ANTIGENS IN FEMALE PATIENTS INFECTED WITH HIV-1 AND IN THEIR SERONEGATIVE REFERENTS.

TESTS	HIV infected (N=23)	Referents (N=73)	P value
typhi(O9)	8.70 SD 2.31	9.53 SD 6.88	0.5684
para(O4)	8.70 SD 2.31	8.22 SD 1.32	0.2168
Asta	2.15 SD 0.21	2.08 SD 0.24	0.1476
VDRL	7.65 SD 19.21	0.44 SD 2.63	0.0022
TPHA	31.30 SD 79.05	2.19 SD 13.15	0.0030
Asot	303.39 SD 164.81	262.02 SD 169.46	0.4971

KEY:

typhi = Salmonella typhi(O9)

para = Salmonella paratyphi B(O4)

Asta = antistaphylolysin

Asot = anti-streptolysin O

TABLE 7. ANTIBODY TITRES (RECIPROCAL OF SERUM DILUTIONS) TO DIFFERENT ANTIGENS IN MALE PATIENTS INFECTED WITH HIV-1 COMPARED TO THEIR REFERENTS.

TESTS	HIV positive (NO=50)	HIV-seronegative (NO=89)	P value
typhi	9.60 SD 5.11	10.65 SD 15.99	0.6522
para	13.44 SD 23.72	10.34 SD 9.12	0.2733
Asta	2.83 SD 1.58	2.34 SD 1.28	0.0501
VDRL	4.16 SD 12.44	2.15 SD 9.09	0.2783
TPHA	20.80 SD 62.20	8.99 SD 35.03	0.1539
Asot	379.56 SD 325.75	0.33 SD 409.61	0.9914

KEY:

typhi = Salmonella typhi(O9)

para = Salmonella paratyphi B(O4)

Asta = antistaphylolysin

Asot = anti-streptolysin O

TABLE 8. MEAN IMMUNOGLOBULIN LEVELS IN GRAMS PER LITRE IN FEMALE PATIENTS WITH HIV-1 INFECTION/AIDS AND IN SERONEGATIVE REFERENTS.

Ig class	Patients (N=23)	Referents (N=55)	P value
IgG	33.33 SD 10.93	25.09 SD 8.57	0.001
IgM	4.17 SD 2.51	3.43 SD 2.29	0.403
IgA	4.59 SD 2.43	3.32 SD 1.55	0.008

TABLE 9. MEAN IMMUNOGLOBULIN LEVELS IN GRAMS PER LITRE IN MALE PATIENTS WITH HIV-1 INFECTION/AIDS AND IN SERONEGATIVE REFERTENTS.

Ig class	Patients (N=43)	Referents (N=73)	P value
IgG	34.09 SD 11.77	25.33 SD 11.12	<0.0001
IgM	4.63 SD 2.48	3.89 SD 2.78	0.3084
IgA	4.66 SD 2.15	3.86 SD 2.07	0.0264

TABLE 10.PRESENTING DIAGNOSES AMONG FEMALE HIV-1 INFECTED PATIENTS AND HIV-1 SERONEGATIVE REFERENTS

DIAGNOSIS	HIV-1 STATUS		
	HIV-1 POSITIVE	HIV-1 NEGATIVE	TOTAL
Anaemia	-	1 9 (26.0%)	19
Malaria	3 (13.0%)	4 (5.50%)	7
Tuberculosis	6 (26.1%)	11 (15.1%)	17
P.U.O.	1 (4.3%)	1 (1.40%)	2
Hypertension	-	6 (8.20%)	6
Immunodeficiency	7 (30.0%)	4 (5.50%)	11
Lobar pneumonia	1 (4.3%)	6 (8.40%)	7
Gastroenteritis	4 (17.4%)	4 (5.50%)	8
Others	1 (4.3%)	18 (24.6%)	19
TOTAL	23	73	96

TABLE 11. PRESENTING DIAGNOSES AMONG MALE HIV-1 INFECTED PATIENTS AND HIV-1 SERONEGATIVE REFERENTS

DIAGNOSIS	HIV-1 + VE (%) (N=50)	HIV-1 - VE (%) (N=89)	TOTAL
Anaemia	0 (0.0%)	8 (8.40%)	8
Malaria	5 (10.0%)	12 (70.6%)	17
Tuberculosis	11 (22.0%)	24 (26.9%)	35
PUO	1 (2.0%)	3 (3.40%)	4
Hypertension	1 (2.0%)	- -	1
Immunodefiency	- -	3 (3.40%)	3
Pneumonia	1 (2.0%)	- -	1
Gastroenteritis	- -	4 (4.50%)	4
Others	31 (62.0%)	35 (39.3%)	66

TABLE 12. POSITIVE RESPONSES TO A QUESTION ON ANTIBIOTICS USE 1 MONTH PRIOR TO ADMISSION TO HOSPITAL IN HIV-1 INFECTED AND HIV-1 SERONEGATIVE REFERENTS

	HIV-1 SEROPOSITIVE	HIV-1 SERONEGATIVE
ANTIMICROBIALS	(N=73)	(N=162)
Tetracycline	24 (32.9%)	2 (1.2%)
Chloramphenicol	1 (1.4%)	-
Penicillin	7 (9.6%)	10 (6.2%)
Erythromycin	3 (4.1%)	1 (0.6%)
Ampicillin	1 (1.4%)	-
Cotrimoxazole	-	1 (0.6%)
Strepto/thiazina	6 (8.2%)	12 (7.4%)
Chloro/Penicillin	2 (2.7%)	2 (1.2%)
Tetra/Chloro/Pen	18 (24.7%)	10 (6.2%)
Tetra/Pen	4 (5.5%)	3 (1.9%)
Did not use	7 (9.6%)	120 (74.1%)
TOTAL	73 (100%)	162 (100%)

KEY:

Strepto = streptomycin Chloro = chloromphenicol

Tetra = tetracyclin Pen = penicillin

6. DISCUSSION

One of the broad objectives of the study was to compare the antimicrobial susceptibility patterns of some bacterial isolates from HIV-1 infected/AIDS patients with some bacterial isolates from sex and age matched HIV-1 antibody negative controls. Many variables influence the results of antibiotic sensitivity tests, but there are no generally agreed methods or points of reference at present. In this study methods recommended by the international collaborative study were used [92]. The use of percentage as a measure of the difference in sensitivity patterns of the isolates to different antibiotics was not used in this study, because some of the antibiotics were fully effective against the isolates from both the HIV-1 infected and the HIV-1 seronegative referents. The staphylococcal isolates from the HIV-1 seropositive patients showed a higher tendency of "resistance" to chloramphenicol and doxycycline than those isolated from HIV-1 antibody negatives possibly because patients infected with HIV-1 tended to use antibiotics more often than HIV-1 seronegative referents because the former are more prone to frequent infections which need treatment with antibiotics. There was significant difference in mean inhibition zones to vancomycin shown by the staphylococcal isolates from HIV-1 positive and HIV-1 negative controls ($P<0.001$). Despite the difference in sensitivity patterns to vancomycin shown by the staphycoccal isolates from both groups, they were

sensitive to this antibiotic indicating that staphylococci are very sensitive to this antibiotic. There was some difference in sensitivity patterns from the nasal isolates from HIV-1 seropositive patients and HIV-1 seronegative referents to oxacillin, clindamycin, erythromycin, fucidin, cotrimoxazole, gentamicin, netilmicin and cefuroxime. Fucidin, netilmicin and cefuroxime are antibiotics which are rarely used in Tanzania and no resistance was noted to them from the isolates. Surprisingly resistance to sulfamethoxazole/trimethoprim was not noted considering the wide use of this drug in Tanzania (77). It is known also that staphylococci are poorly susceptible to sulphonamides. Probably the synergistic effect of the combination of sulfamethoxazole and trimethoprim explains this finding. However nasal isolates of staphylococci from HIV-1 infected patients, were less sensitive to this drug than those from HIV-1 seronegative referents as expected. In some studies penicillin resistant staphylococci appeared within 3 years of its wide spread use [75-77]. Whether this resistance was due to beta-lactamase production or not was not mentioned. 90% of nasal staphylococcal isolates in this study were beta-lactamase producers meaning that they can inactivate beta-lactam antibiotics. The finding in this study suggests that HIV-1 infected patients harbour staphylococci which are multi-drug resistant as normal skin flora. The isolation rate of coagulase positive staphylococci was significantly higher in the HIV-1 infected/AIDS patients than in HIV-1 seronegatives, a finding which is in agreement with studies done else where [55,61]. Thirty seven percent of the staphylococcal nasal isolates

from HIV-1 infected patients were coagulase positive implying that they were Staphylococcus aureus strains. HIV-1 infected patients tend to harbour pathogenic staphylococci more than HIV-1 seronegatives. This could be the reason why HIV-1 infected patients are more prone to frequent attacks of multiple abscesses, pyomyositis and skin infection by pathogenic staphylococci [64, 66].

Isolates from urine samples from HIV-1 seropositive patients were resistant to 4 of 7 antibiotics tested compared to 1 antibiotic in HIV-1 seronegative referents. Some drugs are excreted through the renal route unaltered. Thus organisms inhabiting the urethral meatus are exposed to those drugs probably at substandard doses therefore with time may induce resitance to them. This is more likely to occur in patients infected with HIV-1 who receive several antibiotics due to repeated infections as it has been shown in this study. The finding that urinary infection was found to be more in those who were HIV-1 seronegative is difficult to explain but may have been due to the small sample size.

In Tanzania there are no published results on immunoglobulin profiles in patients infected with HIV-1 to date. However one such study reported mean levels of IgG, IgM, IgA as 18.55, 2.51 and 2.51g/L in healthy adults respectively [26]. Another study also in healthy adults reported IgG, IgM and IgA mean levels as 18.24, 1.86, and 2.12g/L respectively [27]. In this study immunoglobulin levels of patients infected with HIV-1 and HIV-1 seronegative referents were determined. It is known from studies elsewhere that in AIDS patients there is a

polyclonal B-cell activation resulting in hypergammaglobulinaemia [50], no such study has so far been undertaken in Tanzania. In this study immunoglobulin levels (IgG and IgA) were found to be highly elevated in the study group of HIV-1/AIDS seropositive patients. The immunoglobulin levels in HIV-1 seropositives in this study were 28.30g/L, 4.04g/L, and 4.12g/L for IgG, IgM and IgA respectively, being considerably higher than those of healthy Tanzanian population [26, 27]. The Ig levels in HIV-1 seropositive patients were significantly higher ($P<0.001$) than those of HIV-1 seronegative referents. Ig levels of HIV-1 seropositives being higher than those of HIV-1 seronegative referents indicates that HIV-1 seropositive patients have the tendency of having higher Ig levels than patients with other infections. AIDS patients showed considerably higher levels in IgG and IgA ($P<0.001$) but not significantly higher ($P=0.239$) in IgM compared to patients with tuberculosis. One study reported that IgG and IgA were the most frequently elevated isotypes in 49% and 65% of AIDS patients respectively while higher levels of IgM were noted in only 24% of the AIDS patients [66]. This finding has been reported and has been interpreted as evidence of intact humoral responses in patients with AIDS [3,4,72]. The absence of significant elevation in IgM levels could be attributed to its being the first immunoglobulin isotype class to be elevated in new infections. IgM levels then wane and become substituted by other immunoglobulin classes if infection continues. Of interest is that the immunoglobulin levels in healthy and HIV-1 seropositive patients are much higher in people living in the tropics. This is propably due to

repeated exposure to the many subclinical and clinical infections in the tropics, thus making the immune system to be constantly stimulated to produce antibodies. If this is the case, then, for immunoglobulin profiles to be of diagnostic value in the tropics, an extensive study should be carried out to establish immunoglobulin values for normal and diseased tropical inhabitants.

There was significant (P=0.003) prevalence of antibodies to T pallidum, and to staphylolysin in patients infected with HIV-1 than their referents. The significant prevalence of antibodies to T. Pallidum noted in HIV-1 infected patients were likely to have had infection or a result of exacerbation of old infection due to immune suppression caused by HIV-1 infection. Another reason is that it emphasizes that the mode of transimission of HIV-1 is mostly through sexual intercourse which may lead to aquisition of both HIV-1 and T. pallidum.

Most normal human sera contain small amounts of antibodies to alpha-toxin and Panto - Valentine leucocidin, but the amounts of these antibodies become greatly increased in severe or deep seated staphylococcal infections such as osteomyelitis and their levels may be measured for diagnostic purposes. The diagnostic methods for detection of antibodies of S. aureus in human serum are inadequate. It should be noted that the antistaphylolysin (Astaph) test gives so many non-specific results, especially in patients with joint complaints. The elevation of Astaph in sera of HIV-1 infected individuals seen in this study could be due to deep staphylococcal infection such as abscesses which are common

in these patients.

Infection with Salmonella sp. has been reported extensively in HIV-1 infected individuals else where [50-56]. However, in this study there was no significant difference in the prevalence of antibodies to salmonella antigens (O4 and O9) in the HIV-1 infected individuals and HIV-1 seronegative referents. During AIDS there is a failure to diagnose many of the infections seen in this group of patients serologically may be because of the B-cell abnormality [14]. In this study this finding was not noted.

Tuberculosis has been extensively studied in HIV-1 infected patients and has been reported to be significantly common in this group of patients [96-99], a finding which does agree with this study in females who were HIV-1 infected. The apparent low prevalence of tuberculosis in HIV-1 infected male patients seen in this study could be attributed to the mode of diagnosis which was mainly clinical without supporting radiological or bacteriological findings. It should also be noted that before the HIV-1 epidemic, developing countries achieved at best an annual decline of 3-5% in tuberculosis as evidenced by notification and surveillance of the annual risk of infection [96-99]. With the potential impact of AIDS on the tuberculosis situation in developed and developing contries, more effort should be put on tuberculosis case finding and treatment.

Mean ASOT were markedly elevated in both the HIV-1 infected and in the HIV-1 seronegatve referents, the mean titres being 288 and 343 Todd units respectively. The interpretation of ASOT is influenced by many

streptococcal infection, and individual's ability to respond immunologically to the toxin, must be taken into account, because no set "normal" titres has been established [100]. The relevance of the 200 Todd units referred to as cut off between healthy (below this level) and recently infected (above the level) may or may not be appropriate in Tanzania. Most healthy adults (99%) have ASOT of 125 Todd units [100]. A rise in ASOT of at least 30% over the previous level is usually regarded as reliable. In this study previous ASOT of the patients were not looked for but they could be of use. However in this study no significant difference existed (P=0.991) between ASOT levels in HIV-1 infected patients and HIV-1 seronegative referents [100].

The present study suggests that HIV-1 infected/AIDS patients are infected with normal pathogens more frequently than HIV-1 seronegative patients. It also suggests that HIV-1 infected patients tend to harbour bacterial flora which are multiply resistant to antibiotics. HIV-1 infected/AIDS patients also tend to have higher initial immunoglobulin levels of IgG, and IgA isotypes than non HIV-1 infected patients.

7. CONCLUSIONS

This study suggests that HIV-1 infected/AIDS patients are prone to infections with the usual common pathogens than HIV-1 seronegative controls. Therefore vigorous treatment and prophylaxis should be considered against infection with such pathogens. If prophylaxis is decided e.g. for skin infections which are common in HIV-1 infected individuals then topical application should be considered with non systemic antibiotics, for example bacitracin. And when systemic prophylaxis is required then a narrow-spectrum antibiotic such as cloxacillin should be preferred.

However indiscriminate use of antibiotics increases the appearance of multidrug resistant pathogens as shown in this study and should be avoided when prophylaxis is considered. Therefore prior to use of antibiotics against any pathogens especially in HIV-infected/AIDS patients, a sensitivity pattern of the causative organism should be ascertained; since no good purpose is served and indeed harm may be done if the clinician is encouraged to treat his patients with drugs which are active against bacteria irrelevant to the infection. This may be impractical considering the paucity of trained personnel and diagnostic facilities prevailing in the third world countries. Probably treatment centres for immunocompromised and chronically ill patients should be

considered so that the available little resources could be used. Strong restriction which exist in controlling antibiotic distribution and their use should be strengthened. Antibiotics if used judiciously, almost always would maintain their effectiveness as exemplified in the treatment of tuberculosis and leprosy.

Staphylococci are very sensitive to vancomycin as shown in this study in agreement with findings else where. Nevertheless this agent has become a treatment of choice in Europe for methicillin-resistant S. aureus. Agents with this type of potential should then be recommended in immunocompromised patients. For us in the developing countries our choice of antibiotics should be based on the potency and activity against a wide variety of pathogens. Old terms like broad spectrum antibiotics are meaningless because there is no cut off point for the term. Antimicrobial agents for instance ampicillin which was previously regarded as "broad spectrum", is now a narrow spectrum antimicrobial because many organisms have developed resitance to it.

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