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Comparative Study of the Epidemiology and Aetiology of Bloodstream Infections in Hospitalized Adult Patients in Tanzania, Malawi, and Thailand: The Role of Human Immunodeficiency Virus Type 1 (HIV-1) Infection

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ABSTRACT

Before 1995, the frequencies of mycobacterial and fungal bloodstream infections (BSI) in human immunodeficiency virus (HIV)-infected populations in sub-Saharan Africa and Southeast Asia were unknown. Therefore, a prospective survey of febrile (oral temperature ≥38°C or axillary temperature ≥37.5°C) adult patients who presented to sentinel teaching hospitals in Tanzania (1995), Thailand (1997), and Malawi (1997 dry season and 1998 wet season) was conducted. The objectives were to (I) determine the aetiology, prevalence, and clinical correlates of BSI; and (ii) characterise the role played by HIV infection. After informed consent, a detailed history was recorded for each patient followed by physical examination. Next, blood was cultured for bacteria, mycobacteria, and fungi, and tested for HIV and malaria. Data were collected for 517 patients in Tanzania, 246 in Thailand, and 471 in Malawi. Respective BSI, HIV, and malaria parasitaemia rates were: Tanzania: 28%, 55%, 9.5%; Thailand: 48%, 74%, 0; Malawi dry season: 30%, 74%, 4%; Malawi wet season: 28%, 73%, 31%. The most frequently isolated bloodstream pathogens were *Mycobacterium tuberculosis* (MTB) and non-*typhi Salmonella* species (NTS) in Tanzania; MTB and *Cryptococcus neoformans* in Thailand; MTB and *Streptococcus pneumoniae* during Malawi dry season; and MTB and NTS during Malawi wet season. In each country, HIV-infected patients were significantly more likely to acquire BSI; all patients with mycobacteraemia were HIV-infected. The Malawi findings are the first description of seasonal variation in the occurrence of *S. pneumoniae* and NTS bacteraemias. Logistic regression models yielded predictors of BSI in Thailand (HIV infection, chronic diarrhoea, lymphadenopathy, or splenomegaly) and Malawi (HIV infection, chronic fever, oral candidiasis, or acute diarrhoea). In populations with high prevalence rates of HIV infection, MTB has become the

foremost cause of documented BSI. Similar season- and country-specific surveys, performed periodically in HIV-endemic regions will provide data on the aetiology and predictors of BSI, and facilitate empirical therapy of febrile illnesses.

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STATEMENT OF INVOLVEMENT AND ACKNOWLEDGMENT

This series of studies would not have been possible without the participation of the patients and relatives, and the cooperation and support of medical and nursing personnel at each of the three study sites. To them I am indebted and eternally grateful.

I designed the series of studies to characterize and evaluate the value of blood cultures in the investigation of febrile patients in Africa and Asia. I planned the studies and wrote the study protocol, which was approved by the Ministries of Health in Tanzania, Malawi, and Thailand, and by the Institutional Review Board (IRB) at the Centres for Disease Control and Prevention (CDC), Atlanta, Georgia. I conducted each study onsite, saw and enrolled each patient, personally obtained informed consent and a detailed history from each patient (with translators, where necessary), performed all the physical examinations, personally drew blood from each patient for culture and other blood tests, and processed them in the local laboratory as described. In addition, I personally analysed the generated clinical, epidemiologic, and microbiologic data using univariate and multivariate statistical methods.

I am indebted to Dr. L. Barth Reller for giving me the opportunity to train in medical microbiology at Duke University Medical Centre, for supporting these studies and suggesting that I submit my research towards the Doctor of Medicine thesis, and for his supervision; to Dr. William R. Jarvis at CDC for encouraging me to conduct these studies because of their potential public health and clinical impact; and to Dr. Geoff Scott of University College London Hospitals for agreeing to be my London supervisor and for meticulously reviewing the manuscript and providing important suggestions and critique.

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CHAPTER 1

OVERVIEW

The presence of living microorganisms in blood has substantial clinical importance. From the diagnostic standpoint, a positive blood culture yielding a clinically important microorganism represents either a failure of host defences to contain an infection at its primary focus or failure of the physician to effectively eradicate, drain, excise, or otherwise remove that focus of infection. (Reller, 1997).

Blood is a very special juice. (Goethe, 1749-1823)

Pathogens normally regarded as 'non-opportunistic' have been well documented as causes of systemic infections in human immunodeficiency virus type 1 (HIV-1)-infected patients in industrialized countries [Schrager, 1988; Krumholz et al., 1989]. The presence of an infection in the bloodstream of an individual is an indication of the failure of the host's defences to contain an infection at its primary site, or may also be the result of the failure of a health professional to properly diagnose or treat the focus of infection. Delay or inability to diagnose a bloodstream infection may lead to significant morbidity or mortality. The misdiagnosis of bloodstream infections is common in the developing world due to shortage of skilled personnel, lack of training facilities, shortage of medical supplies, and or the inappropriate use of available resources.

For hospitals in less-industrialized countries, infectious diseases remain the leading cause of death [Hinman, 1998; Mabey et al., 2004]. Thus, it is essential that clinicians in these countries be provided reliable information regarding the most likely pathogens that are

associated with commonly diagnosed infections, and the susceptibilities of these pathogens to available antimicrobials. Without effective microbiology services this is not possible and decisions to treat infections often have to be based on presumptive diagnoses. Unfortunately, development, implementation, or enhancements of basic microbiology services have not been priorities in many less-industrialized countries, particularly in sub-Saharan Africa. In these regions, where many laboratories lack resources, skilled personnel, and diagnostic microbiology services, or where available resources are inappropriately utilized, the nature of various infections in adult inpatient populations remains largely unknown, and the limited available data on the prevalence, aetiology, and epidemiology of putative infections may result in empiric therapeutic options that are either blind or misdirected.

In 1976, before the onset of the HIV-1 pandemic in sub-Saharan Africa, investigators in Ibadan, Nigeria, demonstrated that *Salmonella* species, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were the predominant causes of community-acquired bacteraemia in hospitalized adult patients [Alausa et al., 1977]. Although community-acquired bacteraemia, especially those caused by Gram-positive organisms, had already been shown to be common in HIV-1-seropositive patients in the United States [Eng et al., 1986; Krumholz et al., 1989; Simberkoff et al., 1984; Wimbrey et al., 1986], very little research had been done before 1995 to fully characterize the aetiology and epidemiology of bloodstream infections in adult inpatients in Africa or Asia, especially among those patients with HIV-1 infection.

Although at the start of the 1990s human immunodeficiency virus (HIV) disease was already considered an epidemic in sub-Saharan Africa [Lucas et al., 1993], underlying causes of attributable mortality had not been characterised by representative studies. In 1991,

consecutive adult medical admissions to a large city hospital in Côte d'Ivoire were studied and a sample of patients whose deaths were attributable to HIV infection were further characterised and analysed through autopsies [Lucas et al., 1993]. Of 5401 adult admissions who were evaluated, 50% were HIV-positive; 38% of these HIV-positive patients died. Among 294 autopsies, including 24% of the HIV-positive deaths in hospital, tuberculosis, bacteraemia, and cerebral toxoplasmosis were the underlying causes of 53% of deaths. Tuberculosis was seen in 54% of cadavers with acquired immune deficiency syndrome (AIDS)-defining pathology. In addition, bacteraemia was associated with 16% of deaths in HIV-1-positive patients, in general, and with 7% of deaths among patients with AIDS-defining disease [Lucas et al., 1993]. Because Gram stain rather than blood culture was used to make the diagnoses of bacteraemia, the lack of bloodstream isolates meant that antimicrobial susceptibility testing of bloodstream pathogens could not be done. The predominant bloodstream pathogens ascertained by this method were Gram-negative bacilli. The study, by nature of its design, suffered from selection bias in that death (and by inference autopsies) would have occurred mainly in patients with advanced HIV disease. The major impact of this study, however, was to highlight the importance of bacteraemia and disseminated tuberculosis as the two major underlying pathologies associated with mortality in adult HIV-infected patients in sub-Saharan Africa. The clinical and public health relevance of these findings are underscored by the fact that these two infections are either preventable or treatable.

One of the first major studies of bloodstream infections in an HIV-1-endemic region in Africa was conducted during 1988-1989 in the largest hospital in Nairobi, Kenya [Gilks et al., 1990a]. In this study, consecutive adult patients admitted to the medical service in the

Kenyatta National Hospital were recruited over six months. Following informed consent from study patients, a full medical history, and a physical examination, blood was drawn for culture and HIV-1 testing. The patients were followed up while they remained in hospital. Blood was cultured specifically for bacteria but not for mycobacteria or fungi. Of 506 patients recruited over the study period, 95 (19%) were positive for HIV-1 antibody and the overall bacteraemia rate was 10.1%. The prevalence rate of bacteraemia was 26% among HIV-1-positive patients versus 6% among those patients without HIV-1 infection. *Streptococcus pneumoniae* and *Salmonella typhimurium* were the leading causes of bloodstream infections in the study population, accounting for 68% of the bloodstream pathogens. Moreover, *S. pneumoniae* and *S. typhimurium* were responsible for 25% of all the HIV-1-related medical admissions. *Cryptococcus neoformans* was isolated from only one blood culture. The Nairobi study also demonstrated that the mortality rate among HIV-1-infected patients, who had culture proven bacteraemia, was significantly higher than the rate for study-patients who were not HIV-1-infected (58% vs. 32%) [Gilks et al., 1990a].

Although the Nairobi bacteraemia study yielded important results, there were a few limitations [Gilks et al., 1990a]. First, although 19.2% of the study-population had received antimicrobials in the previous 24 hours before blood was drawn for culture, the investigators speculated but did not conclusively demonstrate any effect of this factor on the bloodstream infection rate. Second, because the study protocol did not utilize a strict case-definition for the selection of study-patients, blood cultures almost certainly were obtained for patients who did not require this investigation in the first place; this probably led to an underestimation of the true bloodstream infection rate.. Third, the investigators did not specifically seek to delineate the frequency of mycobacterial or fungal bloodstream

infections in their study population. And fourth, although that study spanned the 1988 dry season and the 1989 wet season, the design of the study did not enable the investigators to demonstrate seasonal variation in the incidence and prevalence of community-acquired bloodstream infections.

During 1987-1992, the prevalence of bacteraemia in febrile patients aged >8 years was studied in hospitals in Ghana and Kenya. The findings, published in 1995, confirmed the predominance of *Salmonella*, *Klebsiella*, and *Enterobacter* species among bloodstream pathogens [Petit et al., 1995]. However, there were major flaws in the study design. First, although the authors indicated that the study population consisted of paediatric and adult patients, the authors neither indicated the age breakdown of the study populations, nor stratified their main findings by age. In fact, from the design of the study, it was obvious that the authors had concluded, *a priori,* that the epidemiology and clinical characteristics of bacteraemias would be similar in paediatric and adult patients—an incorrect presumption. Secondly, the authors made another presumption that the epidemiology of bacteraemias would be similar in two different African countries—this is not necessarily so. Thirdly, the authors included post-operative patients in their study patient population during the conduct of the study; the relevance of doing this was not clear in their published paper and rendered a selection bias that was not acknowledged in the discussion (e.g., postoperatively, patients may develop fever associated with the actual surgical procedure, anaesthetics, surgical site infection, respiratory tract infection, or basal atelectasis). And fourthly, the researchers cultured blood only for bacteria and not for mycobacteria despite the existing evidence that the latter pathogen was associated with significant morbidity and mortality in HIV-infected patients in Africa [Lucas et al., 1993].

In 1989, investigators in Rwanda sought to investigate and compare the occurrence of bacteraemia, fungaemia, and parasitaemia in newly hospitalized patients using fever (oral temperature ≥38°Celsius) as the case definition for patient recruitment [Taelman et al., 1990]. Of 271 patients who met the fever case definition, 163 (60%) were positive for HIV-1 antibody. The Rwanda investigators reported a bloodstream infection rate of 55%. This rate, however, included the rate of bloodborne malaria parasites, which the investigators had classified as bloodstream pathogens (note: one could have both bacteraemia and parasitaemia at the same time). When the malaria parasitaemia rate was excluded from the analysis (this additional analysis had to be done by the reader and was not part of the published abstract), the true bacteraemia rate was estimated to be approximately 29%. The Rwanda authors concluded that *Plasmodium* species constituted the most common bloodborne pathogen in febrile African adults, regardless of their HIV-1 status. Notwithstanding this biased inference, the overall results of the Rwanda study confirmed the predominance of *S. pneumoniae*, non-*typhi Salmonella* species, and *C. neoformans* as commonly seen bloodstream pathogens in HIV-1-seropositive patients with fever. These data, presented at an international meeting as an abstract in 1990 [Taelman et al., 1990], were never published in the peer-reviewed medical literature.

During 1991, subsequent to the Rwanda study, investigators from the Centers for Disease Control and Prevention (CDC) in Atlanta conducted a study to determine the frequency of nonmalarial bloodborne pathogens and ascertain the predictors of septicaemia and death among adults admitted to the infectious disease ward of a large hospital in Abidjan, Côte d'Ivoire [Vugia et al., 1993]. This study was comparable to the Nairobi study [Gilks et al., 1990a] in that all newly admitted patients, regardless of symptoms, were

recruited, and clinical and demographic parameters (e.g., age, sex, medical history, symptoms, vital signs including oral temperature, and whether the patient was discharged or died) were identical. The laboratory methods for the Abidjan study included HIV-1 testing and blood cultures. The blood culture methods included the Septi-Chek™ (Roche Diagnostic Systems, Montclair, NJ) blood culture bottle for detection of bacteraemia, Trans-Isolate medium for *Neisseria meningitidis, S. pneumoniae*, and *Haemophilus influenzae*, and the BACTEC 13A (Johnston Laboratories, Towson, MD) for recovery of mycobacteria. Of the 319 study-patients that were enrolled, 201 (63%) were positive for HIV-1 antibody and 85 (27%) had positive blood cultures. Of these 85 positive cultures, 31 (36%) were deemed bacterial contaminants. Thus, the true bloodstream infection rate was significantly lower than the published rate (54/319 [17%] vs. 85/319 [27%]; P <0.01). Gram-negative organisms were the predominant bloodstream pathogens with *Salmonella* species most numerous. Ten (3.1%) study patients had mycobacteraemia (eight *M. tuberculosis* complex and two unidentified mycobacterial species); and eight (2.5%) had fungaemia, predominantly *C. neoformans.* The latter finding was consistent with reports that fungal infections associated with AIDS in Central Africa were largely due to *Cryptococcus* species [Desmet et al., 1989; Swinne et al., 1989]. Only four patients had *S. pneumoniae* bacteraemia although previous studies had shown that *S. pneumoniae* is an important bloodstream pathogen in HIV-1-infected patients [Gilks et al., 1990a; Simberkoff et al., 1984; Witt et al., 1987]. The paucity of *S. pneumoniae* in Côte d'Ivoire might have been a reflection of geographic or seasonal variation (that study was conducted during a 4-week period during the height of the Côte d'Ivoire wet season). Of note, neither *N. meningitidis* nor *H. influenzae* were isolated from Côte d'Ivoire patients. In addition, HIV-infected patients were significantly more

likely to acquire bacteraemia, mycobacteraemia, or fungaemia. The most sensitive indicators of the presence of bloodstream pathogens in Côte d'Ivoire patients were an oral temperature ≥38°C or an absolute lymphocyte count <1000 cells/mm^3.

Although studies of bloodstream infections in HIV-1-infected children in sub-Saharan Africa have demonstrated significant mortality in these patients, most of these studies have focused solely on isolating bacteria rather than other opportunistic pathogens, such as mycobacteria or fungi [Wolf et al., 1995; Nathoo et al., 1996; Walsh et al., 2000; Mulholland et al., 1999; Westwood et al., 2000; WHO Young Infants Study Group, 1999]. Gram-negative bloodstream pathogens are common and often predominate, representing 74% of the organisms isolated in a Kenyan study [Musoke et al., 2000]. This predominance of Gram-negative pathogens was observed by Archibald et al. in a study of bloodstream infections in a bacille Calmette-Guérin-vaccinated paediatric population in Malawi, where non-*typhi Salmonella* spp. were the predomonant bloodstream pathogen while Gram-positive microorganisms, fungi, and mycobacteria were uncommon in HIV-infected children [Archibald et al., 2003a]. Gram-negative organisms can be highly invasive and thus are disproportionately important causes of morbidity and mortality [Mulholland et al., 1999; Musoke et al., 2000].

The organisms causing bloodstream infections in children vary and depend on the region in which the study is conducted, the study population selected, and the microbiological techniques used [Mabey et al., 1987; Walsh et al., 2000; Nathoo et al., 1996; Berkowitz, 1984; Hui et al., 1997; Mulholland et al., 1999; Lehmann et al., 1999; Gatchalian et al., 1999; Westwood et al., 2000; Stauffer & Fischer, 2004]. In sub-Saharan Africa, regional differences have also been documented for the frequency and importance of various

microorganisms that cause bloodstream infections in paediatric populations [Walsh et al., 2000; Westwood et al., 2000; Nathoo et al., 1996; O'Dempsey et al., 1994]. In addition, it is possible that the frequency of these microorganisms may be dependent on seasons as has been documented for paediatric patients in The Gambia [Mabey et al., 1987], southern Malawi [Graham et al., 2000] and in Kenya [Musoke et al., 2000]. Way back in 1987, Mabey et al. demonstrated the seasonal occurrence of Salmonella bacteraemia among paediatric patients in The Gambia. More recently, the results of a pilot study of two paediatric inpatient populations in Malawi during each of the 1998 wet and dry seasons indicated that Gram-negative pathogens are the predominant cause of bloodstream infection in both seasons and that Gram-positive organisms, such as *Staphylococcus aureus* and *Streptococcus* spp., are relatively uncommon in this patient population in central Malawi [Archibald et al., 2003b]. The possibility of geographic variation is underscored by the results of a prospective review of all positive paediatric blood culture results from another Malawi hospital over a one-year period, which showed one-quarter of bacteremic episodes were caused by *S. pneumoniae* [Walsh et al., 2000].

With rising rates of HIV-1 infection and concomitant tuberculosis in human populations in developing countries, the probable occurrence of mycobacteraemia in susceptible paediatric populations also needs to be addressed. There are several reasons why only bacteraemia rather then mycobacteraemia or fungaemia has been sought or comprehensively studied in paediatric populations in sub-Saharan Africa. First, although early detection and treatment of bloodstream infection in children generally may improve patient outcomes, routine screening for bloodstream infections among children in less-developed countries might not be feasible because of the unavailability of the financial

or trained personnel necessary to offer or maintain such services. Second, culturing blood simultaneously for bacteria, mycobacteria, and fungi, using conventional methods, often requires drawing >25 ml of blood from adult patients. Drawing such relatively large blood volumes might be impractical, inappropriate or unethical for blood culture research activities involving infants or children, especially those who are very sick, immunocompromised, malnourished, or severely anemic. Third, because of cultural reasons, parents may be reluctant for their children to part with the seemingly large volumes of blood that are necessary when culturing for these groups of pathogens. Fourth, due to the difficulty of drawing blood aseptically from paediatric populations, especially those with malnutrition or small, fragile veins, and low levels of circulating organisms in the bloodstream of children, sensitive methods are needed that can comprehensively detect fungaemia, bacteraemia and mycobacteraemia. In developing countries, hospital personnel, equipment, and finances are limited, often preventing routine performance of blood cultures [WHO Young Infants Study Group, 1999]. For all these reasons, these studies in Tanzania, Malawi, and Thailand did not seek to characterize the epidemiology of bacteraemia, mycobacteraemia and fungaemia in HIV-1-infected paediatric patients. To date, these three categories of bloodstream infections remain uncharacterized for paediatric patients in most most HIV-endemic region in Africa and southeast Asia.

In the United States and other developed countries, *M. tuberculosis* bloodstream infection had been recognised and reported both in HIV-1-seropositive [Barnes et al., 1987; Reimer 1984; Bouza et al., 1993; Shafer et al., 1991] and HIV-1-seronegative patients [Kiehn et al., 1985]. In fact, *M. tuberculosis* bloodstream infections had been reported in 15–56 % of HIV-1-infected patients clinically suspected to have extra pulmonary

tuberculosis [Barnes et al., 1987; Bouza et al., 1993; Shafer et al., 1991; Kramer et al., 1990; Barber et al., 1990; Shafer et al., 1989]. In Brazil, researchers demonstrated that *M. tuberculosis* bloodstream infection is a frequent event in patients with AIDS and suspected disseminated mycobacterial disease [Grinsztejn et al., 1997]. Although the association between HIV-1 infection and tuberculosis had been well documented in Africa [Gilks et al., 1990b; Nunn et al., 1990; Nunn et al., 1992; DeCock et al., 1992], few blood culture studies that included methodology for detection of mycobacteraemia (i.e., disseminated tuberculosis) had been performed there [Kiehn, et al., 1985]. By 1991, *M. tuberculosis* bloodstream infections among HIV-affected patients in Africa was still considered relatively novel enough to warrant being written up as a single case report [Kamamfu et al., 1991].

In Western Europe and North America, *M. tuberculosis* bloodstream infection are relatively less common than *Mycobacterium avium* complex bloodstream infection. Most major studies showed that the latter pathogen is more frequently observed in patients with advanced HIV-1 infection [Askgaard et al., 1992; Barnes et al., 1988; Macher et al., 1983; Reimer 1994. In contrast, *M. avium* complex bloodstream infection appeared to be relatively uncommon in sub-Saharan Africa [Gilks et al., 1990b; Okello et al. 1990]. Researchers in sub-Saharan Africa, who attempted to culture blood specifically for mycobacteria, isolated *M. tuberculosis* in relatively small numbers only [Gilks et al., 1990b; Morrissey et al., 1992; Vugia et al., 1993]. The first published report of disseminated *M. avium* complex infection occurring in patients with advanced HIV-1 infection in sub-Saharan Africa was based on a study of patients with advanced HIV infection in Kenya [Gilks et al., 1995]: among 14 patients with mycobacteraemia, three (21 %) were due to *M. avium* complex and 11 (79 %)

to *M. tuberculosis*. Thus, *M. avium* complex, too, appeared to be uncommon [Gilks et al., 1995] or absent [Morrissey et al., 1992] in HIV-1-infected individuals in these regions.

M. tuberculosis infection is an increasing worldwide public health threat. Approximately one-third of the world's population is infected and *M. tuberculosis* is now responsible for as many as 3 million deaths annually [World Health Organization, 1998]. The burden of active tuberculosis is spread disproportionately among two key regions of the less-developed world: Southeast Asia and sub-Saharan Africa. Of approximately 40 million episodes of active tuberculosis that were estimated to have occurred worldwide in young adults between 1990 and the year 2000, over 28 million occurred in Southeast Asia with another 9 million estimated for sub-Saharan Africa [World Health Organization, 1998].

The HIV-1 pandemic has contributed to the increased spread and mortality from tuberculosis; HIV-1-infected patients are 30 times more likely to develop active tuberculosis and become infectious [World Health Organization, 1998]. Of approximately 20 million persons with HIV/AIDS estimated in the world in 1997, over 5 million were diagnosed in Southeast Asia and 14 million in sub-Saharan Africa [Fox, 1998]. In these regions, where both HIV and tuberculosis infections are prevalent, the hospital setting may play an important role in the spread of *M. tuberculosis*.

Preventing nosocomial transmission of *M. tuberculosis* requires the early clinical recognition of patients with active disease so that isolation precautions may be taken and therapy promptly initiated [Centers for Disease Control and Prevention, 1994]. However, recognition of tuberculosis may be difficult in HIV-1-infected patients; such patients frequently present with atypical signs and symptoms and are susceptible to a wide variety of other pulmonary infections that may mimic tuberculosis and obfuscate the diagnosis [Elliott

et al., 1993; Gilks et al., 1990b; Kramer et al., 1990; Modilevsky et al., 1989; Shafer et al., 1991]. Although simple diagnostic tests, such as chest radiographs [Barnes et al., 1988] and sputum smears [Modilevsky et al., 1989], may assist in the recognition of active disease in hospitalized patients, resources for performing such diagnostic tests are limited in many parts of the less-developed world.

Mycobacteraemia is a key event in the pathogenesis of tuberculosis and may frequently be detected in HIV-1-infected patients with active tuberculosis [Barnes & Arevalo, 1987; Barber et al., 1990; Bouza et al., 1993; Grinsztejn et al., 1997]. However, clinicians in these regions frequently do not suspect underlying mycobacteraemia (a surrogate marker for disseminated tuberculosis) and therefore may not request appropriate diagnostic tests. In addition, lack of or inappropriate use of clinical microbiology resources in hospitals in the less-developed world, or the absence of clinical algorithms for making the decision to obtain *M. tuberculosis* blood cultures from patients suspected to have disseminated tuberculosis can contribute to low detection rates of mycobacteraemia.

Therefore, by 1995, the epidemiologic, clinical, and microbiology features of bloodstream infections caused by bacteria and fungi still remained largely unstudied and uncharacterised in sub-Saharan Africa and Southeast Asia—areas of the world with relatively high rates of HIV infection. Moreover, the prevalence and clinical significance of mycobacteraemia (i.e., disseminated tuberculosis) in these regions was unknown. To address this lack of data on bloodstream infections in patients from these parts of the world, the blood culture study upon which this thesis is based was designed and implemented. The importance of studying bloodstream infections and the role of blood cultures are highlighted and delineated in the following chapters of this thesis.

CHAPTER 2

ROLE OF BLOOD CULTURES IN DETECTING BLOODSTREAM INFECTIONS

A bloodstream infection is defined as the detection of a clinically significant microorganism in the blood. Patients with bloodstream infection may be asymptomatic or symptomatic. Septicaemia is a serious systemic infection characterised by the presence of microorganisms or bacterial products in the bloodstream, together with clinical evidence of a systemic response to infection. Thus, for the clinician, a blood culture is one of the most important microbiological examinations that could be requested for a patient, and the discovery of living microorganisms in a patient's blood has great diagnostic and prognostic importance.

The main portal of entry of bacteria into the bloodstream from extravascular sites is the lymphatic system. With intravascular infections bacteria enter the blood directly. This occurs, for example, with infective endocarditis (acute and subacute), suppurative thrombophlebitis, and infected intravenous or intra-arterial catheters (intravenous fluids, hyperalimentation, dialysis cannulae, and pressure monitors). There are multiple mechanisms for clearing bacteria from the blood; their efficiency depends in part on the microorganism. The liver and spleen play major roles in removal of microorganisms. Capsules and other virulence factors delay clearance, whereas specific antibody promotes removal. Ordinarily a sudden influx of bacteria is cleared from the blood over minutes to hours, except with overwhelming infections or an intravascular focus of infection. The level of microorganisms in an infection represents the balance between bacterial multiplication. invasion of the bloodstream, and the efficiency of the reticuloendothelial system in removing bacteria. The great prognostic importance of bacteraemia stems from its usefulness as index

of failure of the host to localize the infectious process in the extravascular tissues or failure of the physician to remove, drain, or treat an infected focus.

There are three clinical patterns of bacteraemia: transient, intermittent, and continuous. Recognition of the settings in which different types of bacteraemia are likely to occur is helpful in planning a schedule for diagnostic studies and in interpreting results of blood cultures. Transient (minutes to hours) bacteraemia occurs following manipulation of infected tissues (e.g., abscesses, furuncles, and surgical procedures); instrumentation of contaminated mucosal surfaces (e.g., dental procedures, cystoscopy, and sigmoidoscopy); and the onset of bacterial pneumonia, arthritis, osteomyelitis, and meningitis [Everett et al., 1977]. Intermittent bacteraemia occurs most commonly with undrained, occult abscesses within the abdomen or elsewhere. Continuous bacteraemia is a cardinal feature of bacterial endocarditis, both acute and chronic, as well as suppurative thrombophlebitis and infected aneurysms. This pattern also occurs in the first few weeks of typhoid fever and brucellosis; however, compared with endocarditis, very few bacteria per ml of blood are found with these diseases.

There are no specific signs of bloodstream infections or septicaemia, and signs that are present in early septicaemia may be either minimal and/or subtle an may be easily missed with a cursory examination or if the diagnosis of underlying bloodstream infection or septicaemia are not considered. Although the most common clinical features of a bloodstream infection include fever (body temperature >37.8^{0}C [100^{0}F] orally or >38.2^{0}C [100.8^{0}F] rectally), up to 20% of patients with positive blood cultures are afebrile, particularly the elderly, neonates, the immunosuppressed, and the debilitated. An early clinical sign of septicaemia is relative hypotension which may be difficult to interpret if the

patient's baseline blood pressure is unknown. Notwithstanding these limitations, an elevated body temperature has been shown to be a significant predictor of bloodstream infections. A seminal study conducted at Duke University found that patients with a body temperature $\geq 40^{0}C$ were significantly more likely to have positive blood cultures that were clinically significant (*Bacillus* species, coagulase-negative *Staphylococcus* species, diphtheroids, or *Micrococcus* species were invariably found to be contaminants) than patients whose temperatures were normal or only slightly elevated [Weinstein et al., 1997].

The number of organisms present in blood is often low (<1 organism /ml) and cannot be predicted in advance. Thus, low volumes of blood yield fewer positive cultures than cultures of larger volumes. As the volume of blood sampled increases from 2 to 20 ml, the diagnostic yield increases by 30 to 50%. Studies of sequential blood cultures from bacteraemic patients without endocarditis have yielded 80 to 90% positive results on the first culture, 90 to 95% with two cultures, and 99% in at least one of a series of three cultures [Weinstein et al., 1983]. Transient bacteraemia is usually not detected, because organisms are cleared before the appearance of any clinical findings suggesting sepsis. Intermittent bacteraemia presents the greatest challenge because fever spikes generally occur after, rather than during, the bacteraemia. Little is known about the periodicity of bloodstream invasion, except that the bacteraemia is more likely to be present and sustained in the early acute stages of infection. It is generally not useful to collect blood cultures while the patient is receiving antimicrobials.

Since the normal bacterial flora of the skin may be inadvertently inoculated into blood culture bottles, to avoid problems in interpretation of positive cultures the risk of contamination of blood cultures during collection must be reduced to a minimum, ideally

less than 3% [Reller et al., 1982]. Published data suggest that blood culture contamination is minimized when the venipuncture site is cleansed with 70% alcohol followed by 1-2% tincture of iodine or povidone-iodine [Calfee & Farr, 2002]; other data suggest that skin disinfection with tincture of iodine is associated with lower blood culture contamination rates [Strand et al, 1993; Little et al., 1999]. For patients in Tanzania and Thailand, the venipuncture site was disinfected with 70% alcohol followed by povidone-iodine; for Malawi patients, skin disinfection was carried out with 70% alcohol followed by 1-2% tincture of iodine. For maximum effectiveness, the disinfectant should be allowed to dry (1-2 minutes) before blood is aspirated. Each sample of blood (minimum of 10 ml from adults and 0.5-5 ml from infants and children) should be obtained by a separate venipuncture. Most often two blood cultures suffice [Weinstein et al., 1983]; more may be required for certain suspected diagnoses, such as bacterial endocarditis. Multiple bottles filled from a single venipuncture should be interpreted as a single blood culture.

Even with the most exacting techniques of collection and processing, contamination of blood cultures occurs occasionally. Bacterial growth in blood cultures first detected beyond 72 hours of incubation is more commonly associated with contamination than with true bacteraemia; however, prior exposure of the patient to antimicrobials may also delay recognition of positive cultures. Recovery of *Propionibacterium acnes*, *Corynebacterium* species, *Bacillus* species, and *Staphylococcus epidermidis* nearly always means contamination, unless these bacteria are present in multiple positive blood cultures obtained by independent venipunctures [Weinstein et al., 1983; Weinstein et al., 1997].

Thus, no investigation is more important for accurate diagnosis and therapy of septic patients than blood cultures. Without an isolated microorganism therapy remains empirical

and antimicrobial susceptibility testing is impossible. A pair of blood cultures, each with an adequate volume of blood, obtained from separate venipunctures should enable detection of most episodes of bacteraemias and fungaemias in clinical patients.

Primary infections, such as pneumonia, urinary tract infections, or gastrointestinal infections, often result in bloodstream infection and may cause fever. Thus, the management of febrile inpatients may be greatly assisted by the detection of bloodstream infections. On the other hand, although fever is one of the most common clinical features associated with bloodstream infections, it is related to a variety of diseases and infections and therefore does not have adequate specificity on its own for predicting the presence of an underlying bloodstream infection. Because of the limitations in the provision of microbiology services in less-developed settings, and the lack of specificity of fever, it may be useful to be able to predict the presence of bloodstream infections from the clinical features of the illness. Moreover, since blood is a normally sterile site, blood cultures, properly performed, have a high predictive value positive for bloodstream infections.

CHAPTER 3

MATERIAL AND METHODS

This project was designed to meet the challenge of comprehensively defining the role of emerging pathogens that cause bloodstream infections in febrile hospitalized patients in three countries with relatively high endemic rates of HIV infection—Tanzania, Malawi, and Thailand—through the conduct of cross-sectional studies with clinical, epidemiologic, and microbiologic components. The studies were conducted in sentinel hospitals in each of these countries. Sentinel hospitals tend to be large institutions (usually >500 beds) that are the main teaching centres for medicine, surgery, nursing, and laboratory science; they commonly house specialized intensive care units (ICU), surgery, haemodialysis, or units for invasive medical procedures; they have problems with hospital infections and antimicrobial-drug resistance; they are associated with microbiology laboratories that are often reference centres with the ability and capacity to conduct various microbiologic tests using scrupulous, quality-controlled methods; and they usually are government affiliated and have very close links with the respective ministry of health.

OBJECTIVES

The overall objectives of the study in each of the three countries were as follows :

(i) To determine the prevalence, aetiology, and clinical outcome of bloodstream infections caused by bacteria, mycobacteria, and fungi in febrile, hospitalized adult patients.

(ii) To determine the prevalence of HIV-1 infection in febrile, adults admitted to hospital.

(iii) To ascertain clinical predictors for bloodstream infection (e.g., enlarged lymph nodes, family history of cough) in this patient population.

(iv) To determine risk factors for bloodstream infection (e.g., HIV-1 infection, Bacille Calmette Guérin [BCG], or nutritional status) in this patient population.

(v) To determine the effect of seasonal variation on the prevalence and aetiology of bloodstream infection.

(vi) To determine the frequency of occult mycobacteraemia and its influence on mortality.

STUDY DESIGN

At sentinel hospitals in Tanzania, Thailand, and Malawi, adult patients with fever were nonselectively recruited and enrolled in the study following informed consent. After documentation of clinical and epidemiologic data, study-patients underwent a comprehensive physical examination. Blood was then drawn from patients and cultured. These blood cultures were processed on site using previously pilot-tested, simple and cost-beneficial methods that could be continued at the study hospitals after the completion of the study. The requirements for microbiologic processing of blood cultures was a laboratory with an incubator, centrifuge, and a refrigerator. During the conduct of the study, local technicians and doctors were trained in microbiologic methods and it was expected that surveillance of bloodstream infections at the study sites would be continued through the initiative of the local investigators at each of the study hospitals. The project was conducted first in Tanzania (Appendix A), then Thailand (Appendix B), and finally in Malawi (Appendix C). The study was completed at each site before it was initiated at another site.

The study protocol was comprised of two integrated components:

(i) *Clinical/epidemiologic* component: This component involved the study of all adult patients who presented to hospital with fever and were admitted to the inpatient medical service or were followed up in the outpatient clinic. All clinical, epidemiologic, and outcome data were recorded in a standardized assessment form specifically developed for this purpose (Appendix D).

(ii) *Microbiology* component: In this component, blood was drawn from study-patients and cultured for bacteria, mycobacteria, and fungi. Several blood culture systems were used with inbuilt redundancy for quality assurance. These systems included commercially prepared biphasic (broth/agar) systems for aerobic bacterial blood cultures and mycobacterial blood cultures, respectively. A lysis-centrifugation system was used in parallel for isolation of mycobacteria, yeasts, and fungi. In Malawi, an additional blood culture system that recovers mycobacteria, bacteria, and fungi from just five ml of blood was introduced. The relative utility of each blood culture system for detection of bacteraemia, mycobacteraemia, and fungaemia was assessed. The frequency of malaria parasitaemia was ascertained and documented, where appropriate. HIV-1 testing was performed on each study patient following pre-test counselling and patient consent. After the results of HIV-1 status became available, the results were made available to each patient with post-test counselling. This was carried out by the patient's physician or a full-time counsellor.

The integrated data were analysed to (i) establish prevalence rates of bloodstream infections, HIV infection, and malaria; (ii) determine associations between clinical and microbiologic variables and bloodstream infections; and (iii) evaluate the effect of acquiring a bloodstream infection on patient outcome (i.e., discharge from hospital or death).

PATIENT ENROLLMENT AND CLINICAL DATA COLLECTION

This was a cross-sectional study of adult patients with fever admitted to the medical wards of sentinel hospitals in three countries—Tanzania, Thailand, and Malawi. In Tanzania and Malawi, the minimum age of admission to the adult medical wards was 14 years; in Thailand, 15 years. Patients were enrolled in the study if they were febrile on admission or if they developed a fever within 12 hours of admission. Fever was defined as an oral temperature ≥38.0° Celsius or an axillary temperature ≥37.5° Celsius. An individual was enrolled once and only one set (i.e., one peripheral blood draw) of blood cultures was drawn at the time of admission to the study. Blood cultures were not drawn each time a study-patient developed a fever.

SAMPLE SIZE

The sample size was based on whether or not the true prevalence of bloodstream infections in a random sample of febrile patients is 25%. This rate was an estimate of the bacteraemia rates in Kenya, Rwanda, and Côte d'Ivoire (10%–24%) [Gilks et al., 1990a; Taelman et al., 1990; Vugia et al., 1993]. However, because these rates did not reflect the frequency of mycobacteraemia or fungaemia, the true bloodstream infection rate was thought likely to be >25%. The population at each of the urban study sites was >1 million. If the true rate is 0.25, then by accepting deviations of 0.25 ± 0.05, the calculated confidence interval of the prevalence rate is 0.30-0.20. To ensure that the true proportion of bloodstream infections in the population will have a 95% probability of falling between 0.20 and 0.30, the sample size

was calculated as follows: sample size = 1.96 × 1.96 [0.25(1– 0.25)] / (0.05) (0.05) = 288. The percentile of the standard normal distribution for a 95% confidence interval =1.96. Thus, at each study site, it was projected that approximately 300 febrile adult patients would have to be recruited and enrolled to correctly estimate the bloodstream infection prevalence rate.

ETHICAL CONCERNS AND INFORMED CONSENT

Participation was voluntary and all patients were informed that they were free to refuse participation or withdraw participation after enrollment in the study without consequence to their medical management or care. If a patient was unconscious or otherwise unable to communicate, the next of kin was asked to provide informed consent. The next of kin was informed that he/she was free to refuse to give consent for participation of the patient in the study without consequence to the quality of medical management or care. If a patient was unconscious and there was no next-of-kin, blood cultures were performed on the patient only if the clinician taking care of the patient at the study hospital made such a request on the grounds that the results might assist in the medical management of the patient; these unconscious patients with no next-of-kin, however, were not be entered into the study. The investigator saw every patient and explained the informed consent form (Appendix E) to each patient or guardian at the initial clinical assessment. The protocol for the studies was approved by the Institutional Review Boards (IRB) at CDC, Atlanta, Georgia, USA and by the Ministries of Health of Tanzania, Thailand, and Malawi.

CONFIDENTIALITY

To protect the privacy of patients participating in the study, clinical information and results of bloods tests and blood cultures were kept in the medical records maintained in the hospital wards. Hard copies of data collection taken to the collaboration field site were kept in a locked room when not in use. Names were removed from all data collection forms. A hospital administrator kept the linkage code between the patient and laboratory results.

HIV-1 TESTING

Patients were advised that HIV-1 testing is voluntary and confidential. They were educated about the increased risk of serious tuberculosis disease in individuals who are co-infected with HIV-1 and *M. tuberculosis*. All patients were offered pre- and post-test counselling.

MEDICAL MANAGEMENT

All decisions regarding patients' medical management and follow-up were made by the physicians responsible for their care at the participating hospital. Clinicians were promptly advised by the investigator about positive blood cultures and the identities of organisms.

COMPLICATIONS OF VENIPUNCTURE

All study-patients were advised that (i) venipuncture is a common diagnostic procedure which aids medical management; (ii) obtaining a blood culture by needlestick puncture and withdrawing blood is a routine medical procedure; (iii) possible complications may include bruising at the site of needle puncture and/or bleeding.

CHAPTER 4

DETAILED STUDY METHODS

The studies were conducted in Tanzania (1995), Thailand (1997), and Malawi (1997 dry season and 1998 wet season). The first study in Tanzania established the methodology for the subsequent Thailand and Malawi phases by demonstrating the feasibility and utility of the proposed microbiologic methods in a less-developed country, and enabling the data collection form to be field tested and refined. This resulted in a short, user-friendly, but comprehensive standardized questionnaire that addressed relevant epidemiologic and clinical variables for the respective study populations. The detailed methods used to conduct the studies in each of the countries follow:

DAR ES SALAAM, TANZANIA

Patients, site, and study period

In Tanzania, the sentinel hospital selected for the study was Muhimbili Medical Centre (MMC), which has >3,000 beds, and is the largest hospital as well as the main medical referral centre in Tanzania. There are on average 25 admissions to the medical service per day; over 50% of these patients are febrile. For each 24-hour period from February 18 through April 16, 1995 (study period), all febrile adults (≥15 years) admitted to the adult Medical Unit in the Mwaisela Block at MMC were seen by the principal investigator. After informed consent, a detailed history was obtained and a physical examination performed. Before antimicrobial treatment was commenced, 25 ml of venous blood was drawn for culture, HIV-1 serology, and malaria parasitaemia testing, after skin cleaning with povidone-iodine and isopropyl alcohol. HIV-1 enzyme-linked immunosorbent assay (ELISA) tests

were repeated on patients with one positive result and confirmed by Western blot at Duke University Medical Center. The ward was notified as soon as an organism was isolated from the blood and again when the organism was identified. Doctors and other ward staff were given advice on antimicrobial regimens when appropriate.

Laboratory

Blood cultures. After blood was drawn up into the syringe, the needle was discarded in a sharps collection box and replaced with a clean needle before inoculation into the blood culture bottles and lysis centrifugation tubes. Following cleansing of the bottle cap rubber diaphragm with 70% alcohol, 10 ml of venous blood were inoculated at the bedside into a Septi-Chek™ biphasic bacterial (SC-B) blood culture bottle (Becton Dickinson Microbiology Systems [BDMS], Cockeysville, MD) to which an agar slide paddle (BDMS) was attached in the laboratory. The The blood culture bottle was then momentarily inverted so that the contents covered the agar paddle. An additional 10 ml of blood was added to an ISOLATOR 10 (Isolator) tube (Wampole Laboratories, Cranbury, NJ) for lysis and centrifugation within 8 hours of venesection. To further minimize the risk of contamination, the rubber stopper of the Isolator tube was cleaned with 70% alcohol before inoculation with blood, immediately after centrifugation, and also before the bung was accessed with the pipette for lysis-centrifugation concentrate withdrawal. A one-ml aliquot of the lysis-centrifugation concentrate was inoculated into a Septi-Chek™ AFB (SC-AFB) biphasic mycobacteria blood culture bottle (BDMS) containing Middlebrook 7H9 broth, to which acid-fast bacilli (AFB) culture supplement (BDMS) was added, and to which an AFB agar paddle (BDMS) was attached in the laboratory.

The remainder of the lysis centrifugation concentrate was inoculated onto Middlebrook 7H11, heated blood (chocolate), and Inhibitory Mold Agar (IMA) slants (BDMS). The bacterial and mycobacterial blood culture bottles and agar slants were incubated aerobically at 35°C. The SC-B blood culture bottles were examined twice for signs of growth in the first 24 hours following incubation, then daily for the next seven days. Broth from bottles that remained clear after seven days were terminally subcultured onto sheep blood agar plates. Preliminary identification of organisms was made in Tanzania using standard microbiologic tests. If an enteric organism was suspected on initial microbiologic analysis, a standardized inoculum of the organism was incubated in the BBL®Crystal™ Enteric/Nonfermenter Identification System (BDMS) for identification according to recommended procedures. All bacterial and fungal isolates were suspended in trypticase soy broth with calf serum and frozen to -70°C. The SC-AFB blood culture bottles were inverted and rotated daily to cover the agar paddles during the first week, and then once weekly for 8 weeks or until growth was observed. Mycobacterial isolates were frozen to -70°C in freezing media (7H9 broth with 10 % sucrose) and also stored on Middlebrook 7H11 agar slants. All frozen isolates were transported to the Clinical Microbiology Laboratory, Duke University Medical Centre, where the identities of all bacteria, mycobacteria, and fungi isolates were confirmed. *M. tuberculosis* complex and *M. avium* complex were identified using AccuPROBE (Gen-Probe, San Diego, CA) DNA probes and biochemical tests.

DNA fingerprinting of M. tuberculosis isolates. M. tuberculosis isolates were characterized by Restriction Fragment Length Polymorphism (RFLP) assays, which are based on polymorphisms generated by variabilities in both the copy numbers and the chromosomal

positions of DNA IS*6110* elements in *M. tuberculosis* complex isolates. The assay used in these studies was a standardized methodology based on testing for repetitive DNA IS*6110* elements in *M. tuberculosis* complex [van Embden et al., 1993]. The standardized technique encompasses the following: growth of *M. tuberculosis*, extraction of DNA, restriction endonuclease digestion, Southern blotting, and probing for the insertion sequence element [van Embden et al., 1993]. The standardized RFLP technique used to characterize the *M. tuberculosis* strains isolated in Tanzania, Malawi and Thailand were identical to that described and published by van Embden et al. [van Embden et al., 1993].

Malaria. Blood films were prepared for a control group of 150 afebrile trauma patients in the main MMC orthopaedic ward; these control-patients had been admitted for trauma, did not have clinical manifestations of sepsis, and were matched for age and sex with randomly selected patients from the febrile study group. For each study patient, one thick and two thin blood smears were stained with Fields stain and examined quantitatively for malaria parasites.

HIV-1 serology. Serum samples were labelled with a randomly generated code number together with the age and sex of the patient. Sera were assayed in batches by ELISA (Vironostika® HIV-1 Microelisa System, Organon Teknika Corporation, Durham, NC) for HIV-1 antibody. Each positive test was repeated using the same type of ELISA test kit and then confirmed by Western blot analysis at Duke University Medical Center. The code was then broken and the ELISA and Western blot results correlated with patients' study records.

Antimicrobial susceptibility testing. Antimicrobial susceptibility tests and interpretations were performed at Duke University Medical Centre, according to the recommendations and guidelines proposed by the National Committee for Clinical Laboratory Standards (NCCLS) [National Committee for Clinical Laboratory Procedures, 1997a & 1997b]. The antimicrobial panel chosen for testing included affordable drugs readily available to patients hospitalized at MMC. Susceptibilities of gram-negative organisms to the selected antimicrobials were tested using the Microscan® Walkaway (Baxter Diagnostics Inc. Deerfield, IL). Other susceptibilities were tested using disk diffusion procedures (Kirby-Bauer test). The E-test® (AB BIODISK, Culver City, CA) was used to test the susceptibility of *S. pneumoniae* to penicillin. Susceptibility testing of *M. tuberculosis* isolates was performed using the BACTEC radiometric method (Becton Dickinson Diagnostics Instrument Systems, Sparks, MD).

BANGKOK, THAILAND

Patients, site, and study period

The study protocol was identical as for Tanzania and was conducted at Bamrasnaradura Hospital, a 500-bed hospital located in the northern part of Bangkok, Thailand. This hospital is one of the largest infectious diseases hospitals in the region and is a major referral centre for HIV-infected patients. Typically, during November through April, there are approximately 10 admissions to the adult medical service per day and more than half of these patients tend to have fever. The hospital microbiology facilities included an automated blood culture system that was adequate for bacterial cultures but did not accommodate mycobacterial blood cultures, and was insensitive for the detection of fungaemia.

For each 24-hour period from February 11 through April 12, 1997 (study period), all adult (≥15 years) patients who met the fever case definition on admission to the medical service at Bamrasnaradura Hospital were seen and evaluated by the principal investigator. Following informed consent, a detailed history was obtained through a translator, after which a comprehensive physical examination was performed. Data obtained included age, sex, medical history of acute and chronic symptoms, antimicrobial therapy before hospital admission, clinical findings, hospital course, and outcome (e.g., discharged or died). Fever and diarrhoea were deemed chronic if present >1 month; chronic weight loss was defined as weight loss >10% of usual body weight. Data were entered on standardized forms. Additional diagnostic tests, such as chest radiographs, sputum smears for AFB, and complete blood counts were requested when deemed appropriate by the attending physician.

Laboratory

The blood culture methods, malaria smear preparation, and all isolation, processing, identification, and confirmatory methods used in Thailand were identical to those used in Tanzania. Aliquots (1 ml) of the Isolator concentrate were inoculated into the SC-AFB blood culture bottles and onto solid media. The identities of uncommon *Mycobacterium* species (e.g., *Mycobacterium simiae* and mycobacteria strains of the SAV Group*) were confirmed using high performance liquid chromatography (HPLC).

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* *Mycobacterium* organisms that resemble *M. avium* complex by conventional biochemical tests but have high-performance liquid chromatography profiles that are more consistent with *M. simiae*.

HIV-1 serology. Serum samples were assayed in batches using two ELISA kits: (1) Enzygnost anti-HIV ½ Plus (Behring AG, Marburg, Germany) and (2) ACCESS HIV ½ (Sanofi Diagnostics Pasteur S.A., Marnes-LA Coquette, France). HIV ELISA tests were repeated if the first test was positive.

LILONGWE, MALAWI (DRY AND WET SEASONS)

Patients, site, and study period

Lilongwe Central Hospital (LCH) is the largest government regional medical centre in central Malawi. It has approximately 500 beds and provides services to a patient catchment area of 230,000 citizens, regardless of their ability to pay. During the dry season, there are approximately 4-8 admissions to the medical service per day and about 50% of these patients have febrile illnesses. Fevers in adult LCH patients are most often attributed to malaria, tuberculosis, diarrhoea, sepsis, or HIV-1 infection. LCH clinicians estimate that 30%–40% of febrile, adult patients receive antimicrobials or antimalarials before admission to hospital. At LCH, <1 % of newly admitted adult patients with fever have blood cultures performed. All patients who present to LCH are triaged in the medical outpatient department where the decision is made either to admit or follow-up the patient in the outpatient department. Patients diagnosed with *M. tuberculosis* disease are referred to a separate sanatorium affiliated with LCH for appropriate treatment and follow-up.

The microbiology facilities at LCH were limited to only Gram stains routinely performed on specimens submitted to the hospital laboratory and the preparation and examination of blood smears for malaria. Thus, many of the above clinical diagnoses (e.g., tuberculosis, diarrhoea, sepsis) were never confirmed by laboratory testing. Cultures of

cerebrospinal fluid and occasional blood cultures for selected patients are performed at the Community Health Sciences Unit laboratory (run and administered by the Malawi Ministry of Health), located approximately one mile from the hospital.

For each 24-hour period from August 20 through September 16, 1997 (study period and height of the dry season in Malawi), all patients who met the fever case definition on presentation or admission to LCH were evaluated. Recruitment methods, informed consent, data collection, and laboratory studies were similar to Tanzania and Thailand. Additional data collected included whether patients were admitted to the wards or managed as outpatients, and whether patients admitted to hospital for therapy survived. Fever and diarrhoea were deemed chronic if present >1 month; cachexia was defined as weight loss ≥10% of usual body weight. Additional diagnostic tests, such as chest radiographs, sputum smears for AFB, and blood counts were requested when deemed appropriate by clinicians.

To ascertain whether the prevalence of bloodstream infections varied by season, the study was repeated at LCH during March 22–May 6, 1998 (study period and height of the Malawi wet season). The clinical and epidemiologic components of this study were identical to those used during the dry season.

Laboratory

The laboratory protocol in Malawi was essentially similar to that used in Tanzania and Thailand except for two differences: (i) ½ ml (instead of 1 ml) aliquots of the lysis-centrifugation concentrate were inoculated into broth and onto solid media, and (ii) a novel but simple microbiologic method for culturing blood comprehensively for bacteria, mycobacteria, and fungi was introduced in the protocol to be used in parallel with the

previous microbiologic methods piloted and used in Tanzania and Thailand. This new blood culture method consisted of inoculation of 5 ml of blood into *BACTEC*™ MYCO/F LYTIC (MFL) blood culture bottles (BDMS).

To ascertain the utility of various blood culture methods that could be adapted for selected use at the Malawi study site after completion of the study, the blood culture methods used during the wet season varied slightly from those used during the dry season. During the Malawi dry season, one MFL blood culture bottle was used; during the wet season, inoculation of blood into the SC-B bottle was discontinued and replaced by inoculation of five ml of blood into each of a pair of MFL bottles.

The MFL bottles were read for evidence of growth using a hand-held ultraviolet (λ = 365 nanometre) lamp to detect fluorescence of the indicator at the bottom of the bottle. All MFL bottles were examined for signs of growth twice in the first 24 hours following incubation, daily for the next 7 days, then once weekly for a total of 8 weeks.

Malaria smears. For both wet and dry seasons, thick smears were prepared from the blood of each patient enrolled in the study. Smears were stained using a modified Wright stain and examined for the presence of any malaria parasites under the microscope. Malaria smear results were correlated with results obtained using the Parasight F (BDMS) on a subset of enrolled patients to confirm results from microscopy. In addition, smears were prepared from the blood of a randomly selected control group of adult patients who were attending the outpatient department for minor trauma, did not have fever, and were deemed unlikely to have an underlying infection.

HIV-1 serology. Serum samples were assayed in batches using the SUDS HIV 1&2 enzyme-linked immunosorbent assay (ELISA) test kits (Murex Diagnostics Inc., Norcross, GA). Positive HIV ELISA results were repeated on site using same type of ELISA kit. Serum samples underwent confirmatory ELISA assays at Duke University Medical Center using another ELISA test kit (Vironostika® HIV-1 Microelisa System, Organon Teknika Corporation, Durham, NC) for HIV-1 antibody. All serum samples underwent further confirmatory Western blot testing at the Centers for Disease Control and Prevention.

Antimicrobial susceptibility testing. The susceptibility testing methods were similar to those used for the organisms isolated in Tanzania and Thailand, and were performed by the same personnel at Duke University Medical Centre.

Seasons and rainfall. In Malawi, the wet season is the period between December and April; the dry season runs from May through November. Records with details of the daily levels of rainfall in Lilongwe during 1997 and 1998 were obtained from a local agricultural company. These rainfall data were obtained from daily on-site measurements taken at a fixed location within 5 kilometres of LCH.

STATISTICAL ANALYSES

All clinical and epidemiology data and results from blood cultures, malaria smears, and HIV-1 serology were recorded in standardized forms. These data were then entered into a computer database and analysed using Epi Info computer software (Version 6.02) [Dean et al., 1996]. The Chi Square test with Mantel Haenszel and Yates's correction and Fisher's

exact test, where appropriate, were used to compare categorical variables. Continuous variables were compared using the Wilcoxon two-sample test. Relative risks (RR), odds ratios (OR), and 95% confidence intervals were calculated. The calculation of the population sample size for the Tanzania study was based on the documented prevalence rate of bloodstream infections in Kenya [Gilks et al., 1990a], and Cote d'Ivoire [Vugia et al., 1993]. The sample size for Thailand and Malawi were calculated using the rates ascertained in the Tanzania study. Multivariate analysis and logistic regression were performed using the PC SAS statistical software [SAS Institute Inc., Cary NC]. The McNemar modification of the Chi Square test and, where appropriate, Yates's correction for small numbers of observations was used to compare the various blood culture systems utilized for detection of bacteraemia, mycobacteraemia, and fungaemia.

BLOOD CULTURE CONTAMINANTS

During the conduct of the studies, each blood culture that was positive for bacteria or fungi was evaluated by the principal investigator. Isolates such as *Bacillus* species, coagulase-negative *Staphylococcus* species, diphtheroids, or *Micrococcus* species were deemed contaminants based on published data [Weinstein et al., 1983].

CHAPTER 5

RESULTS

DAR ES SALAAM, TANZANIA

Over the study period (60 days), 1,425 patients ≥15 years were consecutively admitted to the MMC medical service. Of these, 517 (36%) met the study criteria and were enrolled into the study. No patient refused enrollment into the study. There were 280 (54%) males. The median age of the study population was 38 (range 15-95) years. Over the study period, 15 patients died within the first 6 hours of admission before the investigator was notified or blood could be drawn. One hundred and forty-five (28%) patients had a bloodstream infection. One hundred and fifty-five clinically important organisms were isolated (Table 1). Of the 517 study patients, 282 (55%) were HIV-1 seropositive and of the 145 patients with bloodstream infection, 118 (81%) were HIV-1 seropositive. HIV-1-positive patients were significantly more likely than HIV-1-negative patients to have a bloodstream infection (RR = 3.6; 95% CI = 2.5-5.3; P <0.0001). Ten patients had polymicrobial (i.e., >1 pathogen) bloodstream infections; 8 (80%) of these 10 patients were HIV-1- infected.

Sixty-one (42%) of the 145 patients with bloodstream infection had mycobacteraemia. The median time to isolation of mycobacterial species using the lysis-centrifugation system followed by inoculation into biphasic media was 25 days. Biochemical and gene probing techniques identified one of the mycobacterial isolates as *M. avium* complex; the remaining 60 (98%) were *M. tuberculosis* complex. RFLP analysis of *M. tuberculosis* complex isolates demonstrated 45 different fingerprint designations among the 60 isolates. Fifty-nine (97%) of the 61 patients with mycobacteraemia were HIV-1-seropositive (the *M. avium* complex isolate was also from an HIV-1-infected patient).

M. tuberculosis complex was the most frequently isolated pathogen (43%) followed by non-*typhi Salmonella* species (20%); Table 2 shows the six most frequently isolated organisms. These six organisms alone or in combination accounted for 135 (87%) of the 155 pathogens isolated. Of the 29 non-*typhi Salmonella* species isolates, 14 were *Salmonella enteritidis*, ten were *Salmonella typhimurium,* and five belonged to other *Salmonella* sero-groups. There were four *Shigella* species isolates: three *Shigella flexneri* and one *S. dysenteriae*. The three *S. flexneri* infections occurred in HIV-positive patients; the patient with *S. dysenteriae* was HIV-negative.

The isolation rate for non-*typhi Salmonella* species was significantly higher in the HIV-1-seropositive patients than in the seronegative patients ($P < 0.01$) (Table 1). *S. typhi* was isolated once, from one patient who was HIV-1 negative. There was no difference in the prevalence of *S. pneumoniae* bloodstream infection among HIV-1-positive and HIV-1-negative patients. However, the infections due to other *Streptococcus* species (two group G and one group A) all occurred in HIV-1-positive patients. All 10 *C. neoformans* isolates were from HIV-1-positive patients.

Table 1. Bloodstream pathogens in HIV-1-positive and -negative patients, Dar es Salaam, Tanzania.

Pathogen	HIV status		Relative Risk (95 % CI*)	P-value
	Positive (n=282)	Negative (n=235)		
Enterobacteriaceae				
Citrobacter freundii	0	1	†	
Escherichia coli	7	5	1.2 (0.4-3.6)	NS §
non-*typhi Salmonella*	23	6	3.2 (1.3-7.7)	<0.00001
Salmonella typhi	0	1	†	
Shigella dysenteriae	0	1	†	
Shigella flexneri	3	0	†	
Other Gram-negative bacilli				
Acinetobacter lwoffi	1	0	†	
Alcaligenes xylosoxidans	1	0	†	
Burkholderia cepacia	1	0	†	
Pseudomonas aeruginosa	0	0	†	
Serratia plymuthica	0	1	†	
Stenotrophomonas maltophilia	1	0	†	
Gram-positive organisms				
Enterococcus casseliflavus	0	1	†	
Staphylococcus aureus	5	8	0.5 (0.2-1.6)	NS
Streptococcus pneumoniae	6	5	1.0 (0.3-3.2)	NS
Streptococcus Group A	1	0	†	
Streptococcus Group G	2	0	†	
Yeasts				
Candida lusitaniae	0	1	†	
Cryptococcus neoformans	10	0	Undefined	0.002
Histoplasma capsulatum	1	0	†	
Mycobacteria				
Mycobacterium tuberculosis	57	3	15.8 (5.0-50.0)	<0.00001
Mycobacterium avium complex	0	1	†	
Total number of patients with blood pathogens‡	118	27	3.6 (2.5-5.3)	<0.00001

* CI: confidence interval

† Numbers too small to calculate a test statistic

§ NS: not significant

‡ Ten patients had two organisms isolated from their blood: one with *Shigella flexneri* and *M. tuberculosis*, two with *Salmonella* sp. and *M. tuberculosis*, one with *Salmonella typhimurium* and *S. aureus*, one with *Burkholderia cepacia* and *Salmonella* sp., two with *Salmonella* sp. and *S. pneumoniae*, one with *Citrobacter freundii* and *Serratia plymuthica*, one with *Salmonella* sp. and *C. neoformans*, and one with group A *Streptococcus* sp. and *E. coli*.

Table 2. The organisms most frequently isolated from 145 positive blood cultures.

Organism	**Number (% of all positive blood cultures)**
Mycobacterium tuberculosis	60 (43)
Non-*typhi Salmonella* species	29 (20)
Staphylococcus aureus	13 (9)
Escherichia coli	12 (8.4)
Streptococcus pneumoniae	11 (7.7)
Cryptococcus neoformans	10 (6.5)

Note: These six organisms alone or in combination accounted for 135 (87%) of all pathogens isolated.

Seven (1.3%) of the 517 blood cultures yielded organisms that were considered contaminants (three *Staphylococcus epidermidis*, two diphtheroids, one *Micrococcus* sp., one *Bacillus cereus).* There was no significant difference between the contamination rates of the blood cultures taken from the HIV-1-seropositive (1.8%) and -seronegative (0.9 %) patients. Moreover, there was no significant difference between the contamination rates of blood cultures that yielded significant growth and those that were truly negative.

Forty-nine (9.5%) of 517 study patients had malaria parasitaemia; there was no significant difference when compared to the parasitaemia rate (8%) in the afebrile control group. Five (10.2%) of these 49 patients with parasitaemia also had bloodstream infections caused by non-*typhi Salmonellae* spp. Twenty (41%) of the 49 patients with malaria parasitaemia were HIV-1 positive, suggesting that febrile HIV-1-negative patients were more likely than HIV-1-positive patients to have a positive malaria blood film on admission ($P < 0.05$).

Antimicrobial susceptibility tests suggested that chloramphenicol was the most effective of the available antimicrobials against *S. aureus* and *S. pneumoniae.* Four (40%) of 11 *S. pneumoniae* isolates were resistant to penicillin (i.e., minimum inhibitory concentrations ≥ 2 μg/mL) [National Committee for Clinical Laboratory Procedures, 1997a]. Against *Salmonella* species, chloramphenicol was also the most effective antimicrobial. Fifty-seven (95%) of 60 *M. tuberculosis* isolates were susceptible to ethambutol, isoniazid, rifampicin, and streptomycin; two (3.3 %) were resistant to isoniazid and streptomycin and one (1.7%) was resistant to isoniazid only.

The median age of the 60 patients with *M. tuberculosis* bloodstream infection was 35 (range 15-65) years. At presentation, 37 (62%) of these patients gave a history of one or more

family members with chronic cough, shortness of breath, haemoptysis, chronic symptoms (i.e., cough, diarrhoea, or weight loss ≥1 month), or had clinical signs of pleural effusion, or pulmonary consolidation on physical examination. Only 10 (17%) individuals with mycobacteraemia had sputum smears examined for AFB; the results of these sputum smears were not available. Moreover, the prevalence of chronic symptoms, pleural effusion, consolidation, and lymphadenopathy were similar in patients with and without *M. tuberculosis* bloodstream infection. There were no symptoms or signs that were predictive of the presence of mycobacteraemia. Twenty-three (38%) of sixty patients with *M. tuberculosis* bloodstream infection had no respiratory symptoms or physical signs suggestive of underlying pulmonary pathology. Chest radiographs were not available for the majority of these patients. Twenty-seven (45%) of the 60 patients with *M. tuberculosis* bloodstream infection died before discharge from hospital.

BANGKOK, THAILAND

During the study period, 246 patients met the study entry criteria. One hundred and seventy-one (69.5%) were male. The median age of the study population was 32 (range:15-87) years. One hundred and nineteen (48%) patients had bloodstream infection; 19 (16%) of these had polymicrobial (>1 pathogen) bloodstream infection. One hundred and thirty-three clinically important organisms were isolated from patients (Table 3). Of the 246 study patients, 182 (74%) were HIV-1-seropositive; of the 119 patients with bloodstream infection, 114 (96%) were HIV-1-seropositive.

The most frequently isolated bloodstream pathogens were *C. neoformans* (n=30), *M. tuberculosis* (n=27), *M. avium* complex (n=24), non-*typhi Salmonella* (n=16), *S. aureus* (n=7), *Histoplasma capsulatum* (n=4), *Penicillium marneffei* (n=4), *Candida* species (n=2), *M. scrofulaceum* (n=2), *M. simiae* (n=2), and SAV group mycobacteria (n=2). Two blood cultures grew coagulase-negative staphylococcus yielding an overall blood culture contamination rate of 0.8%. RFLP analysis of *M. tuberculosis* isolates demonstrated 25 different electrophoretic band patterns among the 27 *M. tuberculosis* isolates.

HIV-1-positive patients were significantly more likely than HIV-1-negative patients to have bloodstream infection (RR: 8; 95% confidence interval: 3.4 -18.7; P <0.00001). The frequency of bacteraemia was greater among HIV-1-positive than HIV-1-negative patients (31/182 vs. 5/64, P = 0.07), although this difference did not reach statistical significance. Further, HIV-1-positive patients were more likely than HIV-1-negative patients to have mycobacteraemia (57/182 vs. 0/64; P <0.0001), fungaemia (38/182 vs. 2/64; P <0.001), or polymicrobial bloodstream infection (19/182 vs. 0/64; P = 0.002).

Table 3. Distribution of pathogens causing bloodstream infections, Bamrasnaradura Hospital, Bangkok, Thailand, February-April, 1997.

Pathogen	Patients		P-value
	HIV-positive (n = 182)	HIV-negative (n = 64)	
Enterobacteriaceae			
Escherichia coli	1	2	NS*
Klebsiella pneumoniae	1	0	NS
non-*typhi Salmonella* spp.	16	0	<0.05
Other gram-negative bacilli			
Acinetobacter johnsonii	1	0	NS
CDC† Group 03	0	1	NS
Pseudomonas aeruginosa	1	0	NS
Pseudomonas stutzeri	1	0	NS
Gram-positive organisms			
Staphylococcus aureus	7	0	NS
Streptococcus pneumoniae	0	2	NS
Streptococcus Group A	1	0	NS
Nocardia sp.	1	0	NS
Rhodococcus equi	1	0	NS
Yeasts			
Candida parapsilosis	1	0	NS
Cryptococcus laurentii	1	0	NS
Cryptococcus neoformans	30	0	<0.001
Histoplasma capsulatum	4	0	NS
Penicillium marneffei	4	0	NS
Mycobacteria			
Mycobacterium avium complex	24	0	<0.01
Mycobacterium scrofulaceum	2	0	NS
Mycobacterium simiae	2	0	NS
Mycobacterium sp. (SAV)§	2	0	NS
Mycobacterium tuberculosis	27	0	<0.01
Total number of patients with blood pathogens ‡	114	5	<0.000001

* NS: not significant

† CDC: Centres for Disease Control and Prevention

§ SAV: *Mycobacterium* organisms that resemble *M. avium* complex by conventional biochemical tests but have high-performance liquid chromatography profiles that are more consistent with *M. simiae*.

‡ >one organism was isolated from blood of 19 patients

When patients were stratified by age, those aged 20-38 years were more likely than other age groups to have a bloodstream infection ($P < 0.05$), a likely reflection of higher incidence rates of HIV-1 infection in this age group. In addition, patients who received antimicrobial therapy before hospital admission were more likely to have a bacteraemia versus patients who had not received antimicrobials ($P < 0.05$). However, receipt of antimicrobials before admission was not associated with the presence or absence of mycobacteraemia or fungaemia.

Inpatient mortality was significantly higher in patients with bloodstream infection than in those without bloodstream infection (37/108 vs. 19/124; $P < 0.001$). On stratification by type of bloodstream infection, 43% of patients with bacteraemia, 40% of those with fungaemia, and 26% of those with mycobacteraemia died before hospital discharge.

Next, using logistic regression analysis, clinical predictors were ascertained for bacteraemia, mycobacteraemia, or fungaemia. Febrile, HIV-1-infected patients presenting with *chronic diarrhoea, chills and rigors, an absolute lymphocyte count* $< 1,200/mm^3$, *and a total white blood cell count* $> 13,000/mm^3$ *or* $< 4,000/mm^3$ had a 91% estimated probability of having a bloodstream infection caused by any pathogen. Febrile, HIV-1-infected patients presenting with *lymphadenopathy* and an *abnormal white blood cell count* $> 13,000/mm^3$ *or* $< 4,000/mm^3$ had a 50% estimated probability of having a bloodstream infection caused by *Mycobacterium* species. Any febrile patient with a history of *chronic diarrhoea alone* had a 71% estimated probability of having bacteraemia. A febrile, HIV-1-infected patient with a *history of chronic diarrhoea and findings of splenomegaly and oral candidiasis* on physical examination had a an 81% estimated probability of having a polymicrobial bloodstream infection.

Of 46 patients with fever and chronic diarrhoea, 31 (67%) had bloodstream infection.

Of these 31 patients, 6 (19%) had bacteraemia only, 7 (23%) had fungaemia only, 10 (32 %) had mycobacteraemia only, and 8 (26%) had polymicrobial bloodstream infection. Similarly, of 125 patients with fever, lymphadenopathy, and HIV-1 infection, 76 (61%) had bloodstream infection. Of these 76 patients, 10 (13%) had bacteraemia, 15 (20%) had fungaemia, 37 (49%) had mycobacteraemia, and 14 (18%) had polymicrobial bloodstream infection.

Several pathogens demonstrated significant antimicrobial resistance. All six *S. typhimurium* isolates were resistant to both ampicillin and trimethoprim/sulphamethoxazole (TMP/SMZ); three (50%) of the *S. kunzendorf* isolates were resistant to ampicillin but not to TMP/SMZ. All three *S. enteritidis* isolates were susceptible to both ampicillin and TMP/SMZ; all sixteen *Salmonella* species isolates were susceptible to ceftriaxone and ciprofloxacin. All seven *S. aureus* isolates were resistant to penicillin; two (29%) were resistant to erythromycin and one was methicillin-resistant but susceptible to vancomycin and clindamycin. Of the 27 *M. tuberculosis* isolates, seven (26 %) were resistant to isoniazid; eight (30%), to rifampicin; six (22%) to both isoniazid and rifampicin; six (22%) to streptomycin; and four (15%) to isoniazid, rifampicin, ethambutol, and streptomycin. No *M. tuberculosis* isolate was resistant to pyrazinamide.

MALAWI (DRY SEASON)

During the study period, approximately 375 adult patients, who presented to LCH, were assessed in the LCH outpatient department; 233 (62%) met the case definition of fever and were admitted to the study. The median age of all patients in the study cohort was 31 (range: 18–64) years; 128 (55%) patients were male. The median body temperature was 38.8°C (range: 37.5–40.9°C). One hundred and thirty-two (57%) patients were admitted to LCH for further evaluation and therapy; 101 (43 %) patients were followed up and managed in the outpatient clinic. A clinical diagnosis of malaria was made in 114 (49%) patients at the initial evaluation. The clinical characteristics of all study-patients are summarized in Table 4.

Study patients managed as inpatients and outpatients were similar for median body temperature at presentation, and for rates of HIV-1 infection, bloodstream infection, and malaria parasitaemia. However, compared to study-patients who were followed up as outpatients, those who were admitted had a significantly higher median age (32 vs. 29 years; $P < 0.01$), pulse rate (110 vs. 100 beats per minute; $P < 0.001$), or respiratory rate (32 vs. 26 breaths per minute; $P < 0.001$), a lower median systolic blood pressure (110 mm Hg vs. 120 mm Hg.; $P < 0.001$), and were more likely to be female (68/132 vs. 37/101; $P < 0.05$), or to have altered mental status (25/132 vs. 2/101; $P < 0.01$). Study-patients managed as outpatients were significantly more likely to be male, or to have acute symptoms of chills (63/101 vs. 39/132; $P < 0.01$) or cough (49/101 vs. 35/132; $P < 0.01$).

Table 4. Characteristics of study patients, Lilongwe Central Hospital, Malawi, August-September 1997.

Characteristics	Positive blood cultures		P-value
	Clinical feature present	Clinical feature absent	
Previous antibacterial therapy	42/112	28/121	<0.05
Previous antituberculosis therapy	10/23	60/210	NS*
Acute symptoms (<1 week)			
Chills	23/102	47/131	NS
Cough	21/84	49/149	NS
Diarrhoea	6/23	64/210	NS
Skin lesions	0/4	70/229	NS
Chronic symptoms (>1 month)			
Fever	29/75	41/158	<0.05
Cough	23/60	47/173	0.07
Diarrhoea	3/12	67/221	NS
Skin lesions	4/8	66/225	NS
Cachexia	32/84	38/149	NS
Oral thrush	10/19	60/214	<0.05
Lymphadenopathy	7/25	63/208	NS
Hepatomegaly	4/13	66/220	NS
Splenomegaly	3/10	67/223	NS
Varicella zoster scar	11/19	59/214	<0.01
Bacille Calmette-Guérin scar	54/195	16/38	NS
Abnormal chest physical findings	30/102	40/131	NS
Abnormal central nervous system physical findings	15/27	55/206	<0.01
Positive serology for HIV-1 infection	62/173	8/60	0.001

*NS: not significant

Seventy (30%) study-patients had bloodstream infection caused by bacteria, mycobacteria, or fungi and 173 (74%) were HIV-1-infected (Table 5). The rate of malaria parasitaemia among study-patients was not significantly different from the rate in the afebrile control-patient group (9/233 [4%] vs. 3/45 [7%], P = NS). Seventy-five pathogens were isolated from the 70 patients with documented bloodstream infection; three patients had polymicrobial bloodstream infection (Table 5). *S. pneumoniae* was the most common bloodstream pathogen, representing 34% of all isolated pathogens; *M. tuberculosis* was the second most common pathogen, representing 29% of all isolated pathogens (Table 6). In addition, there was one *M. avium complex*, one *M. simiae*, one *M. bovis,* and two *Mycobacterium* species in the SAV group (*Mycobacterium* spp. that resemble *M. avium* complex by conventional biochemical tests but have HPLC profiles that are more consistent with *M. simiae*). *C. neoformans* was isolated from the bloodstream of only five patients.

Table 5. Relationship between HIV-1 status and patients with bloodstream infection (BSI)

Pathogen	HIV-1 Status		Relative Risk (95% CI*)	P-value
	Positive (N = 173)	Negative (N = 60)		
Any BSI	62 (36%)	8 (13%)	2.7 (1.4–5.3)	<0.01
Mycobacteria	24 (14%)	0	Undefined	<0.01
Bacteria	33 (19%)	8 (13%)	1.4 (0.7–2.9)	NS
Fungi	5 (3%)	0	Undefined	NS
Polymicrobial BSI†	3 (2%)	0	Undefined	NS

* CI: 95% confidence interval

† Two patients each had two organisms (*S. pneumoniae* and non-*typhi salmonella* spp.) and one patient had three organisms (*S. pneumoniae*, non-*typhi Salmonella* spp. and *M. tuberculosis*) isolated from their bloodstream.

Table 6. Distribution of bloodstream pathogens by patient HIV-1 status

Pathogen	HIV status		Relative Risk (CI*)	P-value
	Positive (n = 173)	Negative (n = 60)		
Streptococcus pneumoniae	21 (12 %)	4 (7 %)	1.8 (0.7–5.1)	NS †
Mycobacterium tuberculosis	20 (12 %)	0	Undefined	<0.01
Salmonella spp.§	12 (7 %)	1 (2 %)	4.2 (0.6–31.3)	NS
Gram-negative bacilli ‡	4 (2 %)	2 (3 %)	0.7 (0.1–3.7)	NS
Cryptococcus neoformans	5 (3 %)	0	Undefined	NS
Gram-positive cocci ¶	3 (2 %)	0	Undefined	NS
Mycobacterium simiae/SAV group	2 (1 %)	0	Undefined	NS
Mycobacterium avium complex	1 (0.5 %)	0	Undefined	NS
Mycobacterium bovis	1 (0.5 %)	0	Undefined	NS

* CI: 95% confidence interval

† NS: not significant

§ Includes eight *S. typhimurium*, three *S. enteriditis*, and two *S. typhi*

‡ Includes three *E. coli*, two *Klebsiella* spp., and one *Brucella* spp.

¶ Includes one *S. pyogenes*, one *S. sanguis*, and one *Rhodococcus equi*

Antimicrobial use before attending LCH was common among the 233 study-patients: 94 (40%) reported taking antimalarials; 21 (9%) were already on antituberculosis therapy while 111 (48%) patients reported taking some form of antibacterial therapy. Patients already receiving antibacterial therapy (but not anti-tuberculosis or antifungal therapy) were more likely than those without such a history to have a bloodstream infection caused by *Mycobacterium* species (20/114 vs. 4/119, RR: 5.4; 95% confidence interval: 1.8-14.0; P <0.001). The presence or absence of bacteraemia or fungaemia was not associated with any previous antimicrobial therapy.

On univariate analysis, patients with HIV-1 infection were significantly more likely than those without HIV-1 infection to have bloodstream infection caused by any pathogen or mycobacteraemia (Table 5). When the analyses were stratified by pathogen, *M. tuberculosis* bloodstream infection was more frequently seen in HIV-1-positive than in HIV-1-negative patients (P <0.01); this was not so for other bloodstream pathogens (Table 6).

There was no significant difference in the rate of mycobacteraemia in patients with BCG scars vs. the rate in those without (19/195 vs. 5/38; P = NS). Although patients with pulmonary consolidation on physical examination were about three times more likely than patients without consolidation to have *S. pneumoniae* bloodstream infection (RR: 2.8; 95% confidence interval: 1.2-8.0; P = 0.07), possibly because of the small numbers, this association was not statistically significant at the 0.05 level.

Logistic regression analysis highlighted several physical signs that were independently associated with bloodstream infection (Table 7). Rates of positive blood cultures were 100% (5/5) for those patients with all three independent risk factors (HIV-1 infection, altered mental status, and herpes zoster scar), 47% (15/32) for those with two of these risk factors, 32 %

(43/138) for those with one risk factor, and 12% (7/58) for patients with none of the three risk factors. Moreover, having one or more of HIV-1 infection, altered mental status, or herpes zoster scar was 90% (63/70) sensitive but only 31% (51/163) specific in predicting bloodstream infections.

Table 7. Independent clinical predictors of bloodstream infections (BSI) in febrile adults

Type of BSI	Clinical predictor	Odds Ratio (CI*)	P-value
Any BSI	HIV infection	2.1 (1.4-7.2)	<0 .01
	Altered mental status	3.5 (1.5-8.2)	<0.01
	Herpes zoster scar	2.8 (1.0-8.0)	<0.05
Mycobacteraemia†	Pleural effusion	4.5 (1.1-18.1)	<0.05
	Oral candidiasis	4.4 (1.3-15.6)	<0.05
	Chronic fever	8.7 (1.1-25.8)	<0.001
Bacteraemia	Pulmonary consolidation	2.7 (0.95-8.3)	0.06
Fungaemia	Altered mental status	13.5 (2.1-85.0)	<0.01

* CI: 95% confidence interval.

† Only HIV-1-positive patients had mycobacteraemia; there was no mycobacteraemia among HIV-1-negative patients

Mycobacteraemia rates were 59% (10/17) for those patients with three independent risk factors (pleural effusion, oral candidiasis, or chronic fever), 17% (10/58) for those with two risk factors, 4% (4/110) for patients with one risk factor, and 0% (0/48) for patients with no risk factors; all patients with mycobacteraemia were HIV-1-positive (Table 7). Having >1 of these mycobacteraemia risk factors was 100% (24/24) sensitive and 25% (52/209) specific in predicting mycobacteraemia.

Bacteraemia rates were 33% (5/15) for patients with pulmonary consolidation and 17% (37/218) for those without. Rates of fungaemia were 11% (3/27) for patients with altered mental status and 1% (2/206) for those without this clinical feature.

Outcome data (death or discharge from hospital) were available for 131 (99%) of the 132 study patients that were admitted to the medical service for therapy. Of these 131 patients, 27 (21%) died during hospitalization. Patients with HIV-1 infection were as likely to die during hospitalization as patients without HIV-1 infection (24/102 vs. 3/29, RR: 2.3; 95% confidence interval: 0.7-7.0; P = NS). On multivariate analysis, predictors of inpatient mortality included presence of any bloodstream infection (OR: 3.5; 95 % confidence interval: 1.4-10.0; P = 0.01) or altered mental status (OR: 6.6; 95 % confidence interval: 2.4-18.7; P <0.001).

Radiographs and sputum smears were obtained from a significant number of patients. Of 21 patients with *M. tuberculosis* bloodstream infection, 15 (71%) were inpatients. Eleven (73%) of the 15 hospitalized patients with *M. tuberculosis* bloodstream infection had no test result (i.e., chest radiograph or sputum smear) or previous therapy to suggest active tuberculosis at the time of admission. Chest radiographs performed for five patients with *M.*

tuberculosis bloodstream infection were abnormal; sputum smears prepared for three of these five patients were negative for AFB.

Of the 25 *S. pneumoniae* isolates, 8 (32%) had intermediate resistance to penicillin (minimum inhibitory concentration: 0.1-2.0 mg/ml); all blood isolates were fully susceptible to third-generation cephalosporins and chloramphenicol. Although the frequency of penicillin resistance among *S. pneumoniae* isolated from study-patients who reported exposure to antimicrobial agents before admission to the hospital was similar to that in patients who reported no exposure (3/11 vs. 5/14; P = NS), the small numbers of penicillin-resistant *S. pneumoniae* isolates do not have sufficient power to enable any specific conclusions regarding the relationship between exposure to antimicrobial agents and the prevalence of penicillin-resistant *S. pneumoniae.* All *M. tuberculosis* isolates were susceptible to isoniazid and rifampicin; one isolate was streptomycin-resistant and one was pyrazinamide-resistant. All eight *S. typhimurium* isolates were resistant to gentamicin and TMP/SMX but susceptible to chloramphenicol, the principal antimicrobials available for treating gram-negative infections in LCH.

MALAWI RESULTS (WET SEASON)

During the wet season study period, 238 consecutive, febrile adults were enrolled in the study. The median axillary temperature was 38.9°C (range 37.5-42°C). The median age of study-patients was 29 (range: 14-61) years; 137 (58%) patients were female. Comprehensive clinical characteristics were available for 229 study-patients and are presented in Table 8.

Sixty-seven (28%) study-patients had bloodstream infection; only one of these bloodstream infections was polymicrobial (i.e., >1 pathogen isolated). Non-*typhi Salmonella* species, *M. tuberculosis*, or *C. neoformans* were the three most common bloodstream pathogens, representing 41%, 19%, and 9% of all isolated pathogens, respectively. Only one *S. pneumoniae* was isolated. These results were different compared to those reported for the 1997 dry season (Table 9).

Of the 238 study-patients, 173 (73%) were HIV-1-positive; of the 67 patients with bloodstream infection, 55 (82%) were HIV-1-positive. Malaria smears were prepared for 231 study-patients and 73 afebrile, outpatient adult attendees. The rate of malaria parasitaemia was significantly higher in the study-patient group than in the afebrile group from the outpatients clinic (72/231 [31%] vs. 10/73 [14%], RR: 2.3; 95% confidence interval: 1.2 - 4.2; P <0.01). Malaria was the presumptive diagnosis made by clinicians in 114 (48%) study-patients. Of these 114 patients, 64 (56%) were presumptively treated for malaria despite having negative blood smears. Of these 64 patients, 22 (34%) had bloodstream infection; all but one (*S. pneumoniae*) of these bloodstream pathogens were gram-negative bacteria.

Patients with HIV-1 infection were significantly more likely than those without HIV-1 infection to have *M. tuberculosis* bloodstream infection (Table 10); risk of bacteraemia or fungaemia were not associated with patient HIV-1 status. There was no significant association

between the presence of BCG scars and *M. tuberculosis* bloodstream infection. Logistic regression analysis confirmed that oral candidiasis, chronic fever, and acute diarrhoea were independently associated with bloodstream infection (Table 11).

Table 8. Clinical characteristics of study-patients (N=238), Lilongwe Central Hospital, Malawi, March-May, 1998

Characteristic	Frequency n (%)
History of previous antibacterial therapy	78 (33)
History of previous antituberculosis therapy	21 (9)
History of previous antimalarial therapy	96 (40)
Family history of cough	28 (12)
Acute symptoms (≤1 week)	
Chills	78 (33)
Cough	117 (49)
Shortness of breath	100 (42)
Diarrhoea	78 (33)
Skin lesions	10 (4)
Chronic symptoms (≥1 month)	
Fever	90 (38)
Cough	69 (29)
Diarrhoea	16 (7)
Skin lesions	8 (3)
Cachexia	92 (39)
Physical findings	
Oral thrush	43 (18)
Jaundice	6 (3)
Lymphadenopathy	22 (9)
Hepatomegaly	15 (6)
Splenomegaly	15 (6)
BCG scar present	166 (70)
Abnormal chest physical findings	78 (33)
Abnormal central nervous system findings	47 (20)

Table 9. Distribution of bloodstream pathogens, Lilongwe Central Hospital, Malawi: wet season vs. dry season

Pathogen	Season		P-value
	Wet n (%)	Dry n (%)	
Non-*typhi Salmonella* spp.	28 (41)	11 (15)	<0.001
Mycobacterium tuberculosis	13 (19)	19 (26)	NS*
Cryptococcus neoformans	6 (9)	4 (5.5)	NS
Escherichia coli	3 (4)	3 (4)	NS
Salmonella typhi	3 (4)	2 (3)	NS
Staphylococcus aureus	2 (3)	0	NS
Acinetobacter lwoffi	2 (3)	0	NS
Other fungi †	2 (3)	0	NS
Streptococcus pneumoniae	1 (1.5)	24 (33)	<0.0001
Proteus mirabilis	1 (1.5)	0	NS
Rhodococcus equi	1 (1.5)	0	NS
Other *Mycobacterium* spp.	0	4 (5.5)	NS

* NS: not significant
† One *Cryptococcus laurentii* and one *Candida tropicalis.*

Table 10. Relationship between HIV-1 status and bloodstream infection (BSI), Lilongwe Central Hospital, Malawi, March-May 1998

Pathogen	HIV-1 Status		Relative Risk (CI)*	P-value
	Positive N=173 (%)	Negative N=65 (%)		
Any BSI	56 (32)	12 (18)	1.7 (1.0-3.0)	0.04
Mycobacteria	13 (8)	0 (0)	Undefined	0.01
Bacteria	36 (21)	10 (15)	1.4 (0.7-2.6)	NS†
Fungi	7 (4)	2 (3)	1.3 (0.3-6.2)	NS

* CI = 95% confidence interval

† NS: not significant

Table 11. Independent clinical predictors of bloodstream infections (BSI) in febrile adults, Lilongwe Central Hospital, Malawi, March-May 1998

Type of BSI	Clinical predictor	Odds Ratio (CI*)	P-value
Any BSI (N=67)	Chronic fever	3.1 (1.6–6.0)	<0.001
	Oral candidiasis	2.3 (1.1–4.8)	0.03
	Acute diarrhoea	2.2 (1.1–4.1)	0.02
Mycobacteraemia† (N=13)	Chronic cough	16.4 (2.0–139)	0.01
	Family history of cough	5.9 (1.4–24.5)	0.01
Bacteraemia (N=45)	Chronic vomiting	8.5 (1.8–40)	0.007
	Acute diarrhoea	2.7 (1.3–6.0)	0.009
	Jaundice	7.8 (1.2–51.7)	0.03
	Temperature >38.9 C	2.9 (1.3–6.5)	0.007
Fungaemia (N=9)	Chronic cough	7.4 (1.2–44.2)	0.03
	Neck rigidity	17.1 (2.8–105)	0.002
	Herpes zoster infection	29.1 (1.6–542)	0.02

* CI = 95% confidence interval

† All patients with mycobacteraemia were HIV-1-positive

Bloodstream infections were found in 37% (51/137) of patients with >1 of these clinical features vs. 15% (16/101) of patients without. Thus the predictive positive value for bloodstream infection in patients having >1 of these clinical features was 37%. When the analyses were stratified by specific types of bloodstream infection, HIV-1 infection, history of chronic cough, or a family history of cough emerged as independent risk factors for mycobacteraemia (Table 11).

A history of use of antimicrobial, antituberculosis, or antimalarial agents before hospital admission was available for 221 study-patients. For the week preceding hospital admission, antimicrobial use was documented in 78 (35%) of these patients. Preadmission use of antimalarial and antituberculosis agents were documented in 96 (43%) and 21 (10%) of patients, respectively. There was no association between being on any of these therapies on admission and presence or absence of mycobacteraemia, bacteraemia, or fungaemia.

Outcome data (death or discharge from hospital) were available for 199 (84%) of the 238 study patients. The overall inpatient mortality rate in these patients was 24% (47/199). Inpatient mortality was significantly higher in patients with bloodstream infection than in those without bloodstream infection (22/53 [42%] vs. 25/146 [17%]; P <0.001). When stratified by type of bloodstream infection, 37% of patients with bacteraemia, 36% of those with mycobacteraemia, and 71% of those with fungaemia died in hospital. Although patients with HIV-1 infection were more likely to die during hospitalization than patients without HIV-1 infection (42/148 [28%] vs. 5/51 [10%], RR: 2.9; 95% confidence interval: 1.2 - 6.9; P <0.01), on multivariate analysis, independent predictors of inpatient mortality were presence of any bloodstream infection (OR: 3.2; 95% confidence interval: 1.4 - 7.3; P <0.001), altered

mental status (OR: 4.3; 95% confidence interval: 1.8-9.8; P = 0.0001), or chronic symptoms (OR: 2.7; 95% confidence interval: 1.1 - 6.3; P <0.05).

Of the 28 non-*typhi Salmonella* species that were isolated during the wet season, 15 (53%) were *S. typhimurium*, four (14%) *S. enteritidis*, and nine (32%) were other serotypes. All non-*typhi Salmonella* species and *Salmonella typhi* isolates were susceptible to chloramphenicol and ciprofloxacin. However, among the non-*typhi Salmonella* species isolates, there was 64% resistance to TMP/SMZ and 73% resistance to ampicillin. Resistance to TMP/SMZ and ampicillin among non-*typhi Salmonella* species isolates were not associated with exposure to antibacterial agents before admission to the hospital. All 13 *M. tuberculosis* isolates were susceptible to ethambutol, isoniazid, pyrazinamide, rifampicin, and streptomycin.

The mean monthly rainfall in the 1998 Malawi wet season during which this study was conducted was 118 mm/month. In contrast, the mean monthly rainfall during the 1997 dry season period was <1 mm/month.

CHAPTER 6

SUBSIDIARY STUDIES AND ANALYSES

UNRECOGNISED (OCCULT) MYCOBACTERAEMIA

Background. The clinical and public health importance of *M. tuberculosis* bloodstream infections was highlighted in the overview to this thesis (Chapter 1) and has been underscored by the results from the current study showing *M. tuberculosis* as one of the predominant bloodstream pathogens in HIV-infected patients in Tanzania, Thailand, and Malawi. During the conduct of the current study, it was noted that several of the patients who had mycobacteraemia had neither symptoms nor signs that would have suggested underlying *M. tuberculosis* infection. To add clarity to this observation, subsets of the data from Thailand and the Malawi dry season were selected and analysed. Patients were selected for this subsidiary analysis if they satisfied the following conditions: (i) their blood cultures yielded growth of *M. tuberculosis* only; (ii) they were not taking any form of anti-tuberculosis therapy before admission; (iii) there were no symptoms or clinical signs to suggest underlying active tuberculosis infection that would have substantiated further investigation for tuberculosis infection; and (iv) no abnormal chest radiographs or positive sputum smears were documented on or before the day of admission for those patients who perchance had these investigations performed. The objectives of these analyses were to: (i) compare and contrast the prevalence rates of *M. tuberculosis* bloodstream infection among febrile, hospitalized adult patients from two HIV-endemic countries; (ii) determine whether active tuberculosis in patients with mycobacteraemia is, in practice, frequently unrecognised; (iii) ascertain clinical predictors for *M. tuberculosis* bloodstream infection; and (iv) characterize factors associated with the under-recognition of active tubercular disease in patients with mycobacteraemia.

Case definition. A case of unrecognised, active disease was defined as a blood culture positive for *M. tuberculosis* in any adult patient who was not already receiving anti-tuberculosis therapy on or before the day of admission and who had neither an abnormal chest radiogram nor positive sputum smear on or before the day of admission.

Results. A total of 344 patients (216 in Thailand and 128 in Malawi) were included in this analysis (Table 12). The median age of patients was 33 (range: 18-87) years. Sixty-two percent were male; a greater percentage of patients enrolled in Thailand (68%) than Malawi (48%) was male. Of the 344 patients, 255 (74%) were HIV-1-infected; 34 (10%) had *M. tuberculosis* bloodstream infection.

Clinical predictors of *M. tuberculosis* bloodstream infection included the presence of HIV-1 infection, lymphadenopathy, or chronic (>1 month duration) cough, fever, or weight loss (defined as >10% loss in body weight) (Table 13). When the analysis was limited to patients who were HIV-1-infected, oral thrush, chronic cough, fever, or weight loss remained significantly associated with *M. tuberculosis* bloodstream infection; lymphadenopathy was no longer significantly associated with mycobacteraemia.

Table 12. Baseline characteristics of study-patients, Thailand and Malawi

Characteristic	Overall N=344	Thailand N=216	Malawi N=128	P-value
Median age/years (range)	33 (18-87)	33 (18-87)	33 (18-64)	NS*
Male	208 (61%)	146 (68%)	62 (48%)	<0.001
HIV-1-infected	255 (74%)	154 (71%)	101 (79%)	NS
M. tuberculosis bloodstream infection	34 (10%)	20 (9%)	14 (11%)	NS

* NS: not significant

Table 13. Clinical predictors of *M. tuberculosis* bacteraemia in febrile hospital inpatients

	Mycobacteraemia (%)		Relative Risk 95% CI*	P-value
Clinical predictor	**Present**	**Absent**		
All patients				
HIV-1 infection	34/255 (13)	0/89 (0)	Undefined	0.0006
Oral thrush	14/56 (25)	20/286 (7)	3.6 (1.9–6.6)	<0.0001
Lymphadenopathy	20/133 (16)	14/210 (7)	2.3 (1.2–4.3)	0.01
Chronic cough	19/113 (16)	15/230 (7)	2.6 (1.6–4.9)	0.003
Chronic fever	26/143 (18)	8/200 (4)	4.6 (2.1–9.8)	0.0001
Chronic weight loss	26/162 (16)	8/181 (4)	3.6 (1.7–7.8)	0.0003
HIV-infected patients				
Oral thrush	14/56 (25)	20/197 (10)	2.5 (1.3–4.6)	0.004
Lymphadenopathy	20/129 (16)	14/125 (11)	1.4 (0.7–2.6)	0.3
Chronic cough	19/102 (19)	9/113 (8)	2.3 (1.1–4.9)	0.02
Chronic fever	26/136 (19)	2/79 (3)	7.5 (1.8–31)	0.0015
Chronic weight loss	26/155 (17)	2/60 (3)	5.0 (1.2–20.6)	0.016

*CI: confidence interval

Of the 34 patients with *M. tuberculosis* bloodstream infection, five were already receiving anti-tuberculosis therapy at the time of admission (Figure 1). Of the remaining 29 patients, 13 (45%) had either abnormal chest radiographs (eight patients) or positive AFB sputum smears (five patients) on or before the day of admission. Thus, 16 (55%) patients with *M. tuberculosis* bloodstream infection met the case-definition of unrecognised active disease. Of these, 13 (81%) had acute or chronic cough; three subsequently had chest radiographs (all were normal); and none had preparation and microscopic examination of sputum smears for AFB.

Overall, febrile patients at the infectious disease hospital (Thailand) were more likely than patients at the general hospital (Malawi) to have been on antituberculosis therapy, to have had a recent chest radiograph, or to have had an AFB sputum smear performed on or before the day of admission (Table 14). Febrile patients with *M. tuberculosis* bloodstream infection at the general hospital, where chest radiography and sputum testing was less frequent, were more likely than patients at the infectious disease hospital to meet the case definition for unrecognised active disease (10/14 vs. 6/20, RR: 2.4, 95% confidence interval 1.1-5.0; P <0.04). *M. tuberculosis* blood culture isolates from the infectious disease hospital in Thailand were significantly more resistant to available antituberculosis drugs than isolates from the general hospital in Malawi (Table 15).

Figure 1. Unrecognised active disease among patients with *M. tuberculosis* bacteraemia

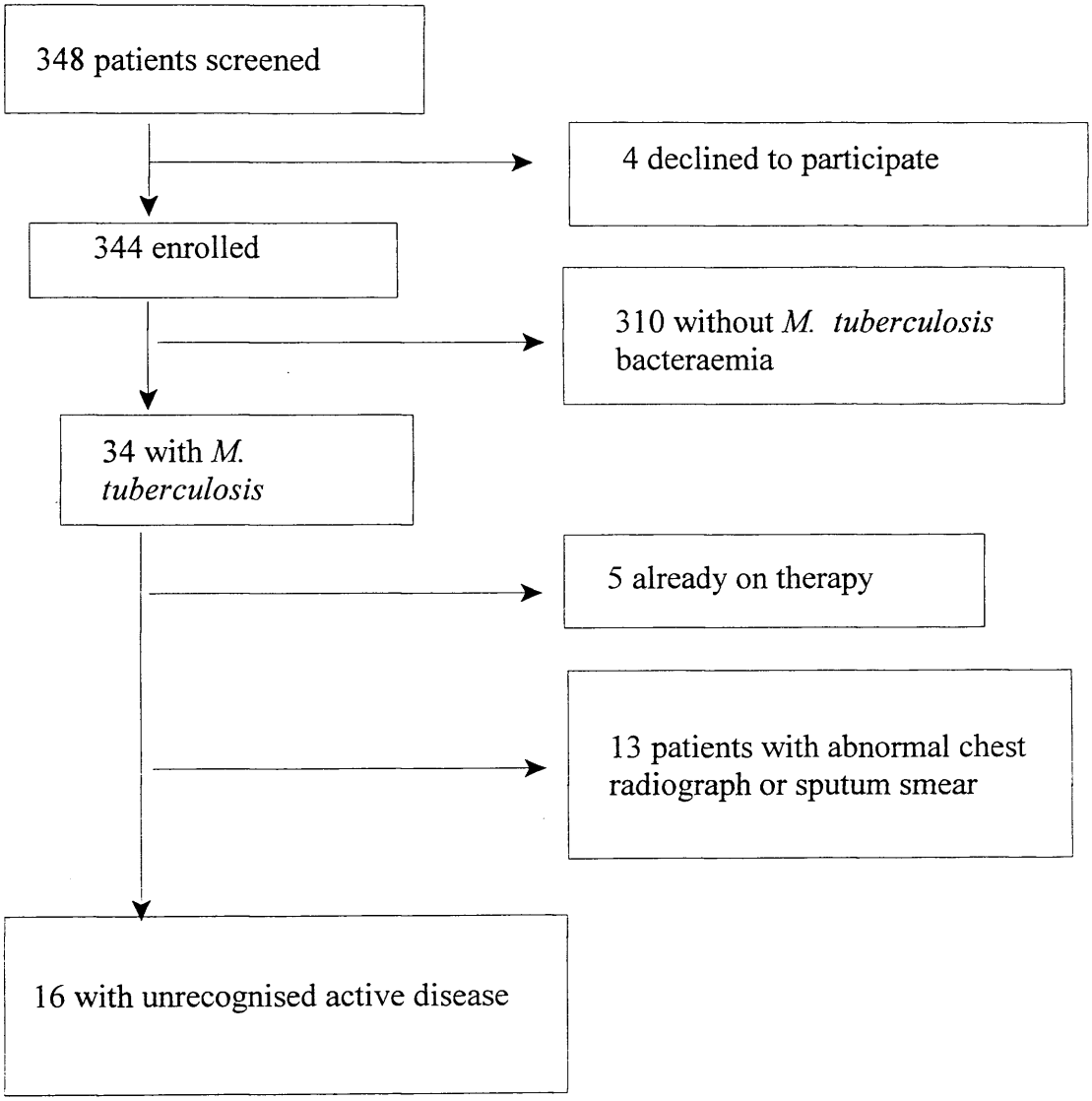

Table 14. Therapy and diagnostic testing on or before the day of admission

	Thailand (n=216)	Malawi (n=128)	P-value
Anti-tuberculosis therapy	40 (18%)	15 (12%)	0.13
Chest radiograph	109 (50%)	16 (13%)	<0.0001
Sputum smear	42 (19%)	5 (4%)	<0.0001

Table 15. Antimicrobial resistance among *M. tuberculosis* isolates from blood, Thailand and Malawi

Antituberculosis Drug	Number of resistant isolates		P-value
	Thailand (n=20)	Malawi (n=14)	
Rifampicin	6 (30%)	0	0.03
Isoniazid	5 (25%)	0	0.06
All other drugs	5 (25%)	2 (14%)	0.7
Multidrug resistant*	5 (25%)	0	0.06

* Resistant to at least isoniazid and rifampicin

COMPARISON OF BLOOD CULTURE METHODS

Background. The results of studies conducted in Tanzania, Thailand, and Malawi suggest that the detection and characterization of bloodstream infections in HIV-1-endemic countries necessitate the culturing of blood for bacteria, mycobacteria, and fungi. This endeavour is made particularly difficult for the following reasons: (i) extensive technician time required to process multiple media and stages (including quality control) to identify the various pathogens; (ii) inadequate microbiology resources or laboratory capacity for processing blood cultures; and (iii) lack of trained staff determined by prohibitive costs for the services of skilled personnel, materials, or laboratory equipment.

In Tanzania and Thailand, where there are high prevalence rates of HIV-1 infection, blood was cultured for bacteria, mycobacteria, and fungi using three blood culture systems simultaneously: (i) the Septi-Chek™ biphasic bacterial blood culture bottle (SC-B); (ii) the ISOLATOR™ 10 (Isolator) lysis-centrifugation system; and (iii) the Septi-Chek™ AFB biphasic mycobacteria blood culture bottle (SC-AFB) containing Middlebrook 7H9 broth. Although using three blood culture systems in parallel would be expected to increase the recovery rate for bacteria, fungi, and mycobacteria, the drawbacks include the relatively large volume (20 to 25 ml) of blood required for recovery of these three groups of pathogens, and increased time, effort, and resources needed for processing.

Recently, the MFL blood culture bottle was introduced and licensed for use in the United States to detect mycobacteraemia [Waite et al., 1998]. Each MFL bottle contains 40 ml of non-selective culture medium (supplemented Middlebrook 7H9 and brain heart infusion broth), specific proteins and sugars, saponin (a blood lysing agent), and added oxygen and carbon dioxide. At the bottom of each bottle, there is a sensor that can detect decreases in

oxygen concentration resulting from growth of microorganisms. Growth of microorganisms may be detected in the automated BACTEC 9000 series systems, or manually by shining the ultraviolet light of a Wood's lamp on the sensor at the bottom of the bottle; the resulting fluorescence is growth-dependent and is proportional to the decrease in oxygen in the bottle.

Methods. In this analysis of the blood culture methods used in Malawi, the MFL blood culture system was compared with the SC-B, the SC-AFB, and the Isolator systems for yield of microorganisms (i.e., bacteria, mycobacteria, and fungi). The dry and wet seasons are termed study periods I and II, respectively. Clinical and epidemiology details and results are described in earlier chapters.

Results. A total of 486 single blood cultures (251 in period I and 235 in period II) were obtained from adult patients admitted to LCH with fever during both study periods combined. Non-*typhi Salmonella* species, *S. pneumoniae,* and *M. tuberculosis* were the most common bloodstream infection isolates. For study periods I and II combined, a total of 8 (1.6%) blood cultures yielded organisms that were considered contaminants, including coagulase-negative *Staphylococcus* species, diphtheroids, and *Micrococcus* species. Four of these contaminants grew in an MFL or SC-B bottle, and four only on the Middlebrook 7H11 agar. For contaminated blood culture bottles, contamination occurred in just four (0.8%) of the 486 blood cultures obtained.

Detection of bacteraemia (study period I). During this study period, the growth of bacteria in 251 blood cultures was assessed using three blood culture systems (MFL, SC-B bottle, and

Isolator concentrate on chocolate agar). From these 251 blood cultures, 44 bacterial pathogens grew in one or more of the three systems (Table 16). Overall, the MFL system had a bacteria recovery rate that was similar to that of the Isolator concentrate inoculated on chocolate agar (34/44 vs. 27/44, P = NS). However, the MFL bottle had a higher bacteria recovery rate than the SC-B bottle (34/44 vs. 24/44; P <0.05).

Detection of bacteraemia (study period II). During this study period, only the MFL bottle was used to detect bacteraemia. The SC-B bottle and inoculation of the Isolator concentrate on chocolate agar were discontinued because their significantly lower diagnostic yield for bacteria did not warrant collection of an extra 10 ml of blood for the former, or the extra work involved in the latter. Of the 235 MFL bottles inoculated during this period, 45 (19%) yielded bacterial growth.

Detection of mycobacteraemia (study periods I and II combined). The growth of mycobacteria was assessed in 486 cultures in each of three systems (MFL, the Isolator concentrate inoculated into the SC-AFB bottle, and the Isolator concentrate on Middlebrook 7H11 agar). From these 486 blood cultures, 37 mycobacterial species were detected by one or more of the three systems. The MFL bottle and the Isolator concentrate in the SC-AFB bottle had similar rates of detection of mycobacteria (30/37 vs. 29/37, P = NS). However, the MFL bottle had a higher rate of isolation of mycobacteria than did the Isolator concentrate on Middlebrook 7H11 agar slants (30/37 vs.15/37, P = 0.002) (Table 17).

Detection of fungaemia (study period I). The MFL system had a higher fungal recovery rate than did the SC-B bottle (3/5 vs. 0/5, P = NS) or the Isolator concentrate on chocolate agar (3/5 vs. 1/5, P = NS); this difference did not reach statistical significance.

Detection of fungaemia (study periods I and II combined). The MFL system had a lower fungal recovery rate than the Isolator concentrate on IMA (6/13 vs. 10/13, P = NS); this difference, however, did not reach statistical significance.

Time of recovery (bacterial and fungal isolates). During study period I, the reading intervals of the SC-B, MFL, and the Isolator concentrate on chocolate agar systems for the detection of bacteraemia, during the first week, were similar. The median time to detect bacteria and fungi was one (range: 1-4) day for the MFL system, one (range: 1-3) day for the SC-B system, and two (range: 1-3) days for the Isolator concentrate on chocolate agar. The median time for detecting fungi by culturing the Isolator concentrate on IMA was 3.5 (range: 3-10) days.

Time of recovery (mycobacterial isolates). Although the reading interval for mycobacteraemia in MFL, SC-AFB, and Middlebrook 7H11 agar cultures were similar during the initial microbiology work-up in Malawi, growth during shipment of these cultures to the United States might have occurred so relative times of detection could not be assessed accurately.

Table 16. Bacterial pathogens isolated from blood cultures, Lilongwe Central Hospital, Malawi, during study period I (1997).

Pathogens	Number of bacterial isolates recovered by:			
	All systems	**MFL* bottle**	**Septi-Chek bottle**	**Isolator concentrate on chocolate agar**
Streptococcus pneumoniae	24	19	17	20
Salmonella spp.†	13	9	4	5
Enterobacteriaceae §	5	5	2	1
Other Gram-positive cocci‡	2	1	1	1

* MFL: MYCO/F LYTIC bottle

† Eight *S. typhimurium*, three *S. enteritidis*, and two *S. typhi*

§ Three *Escherichia coli*, and two *Klebsiella* spp.

‡ One *Streptococcus pyogenes* isolate and one *Streptococcus sanguis* isolate.

Table 17. Mycobacterial species isolated from blood cultures, Lilongwe Central Hospital, Malawi, during study 1997 dry season (study period I) and 1998 wet season (study period II)

Pathogens	No. of mycobacterial isolates recovered by:			
	All systems	**MFL* bottle**	**Isolator concentrate in Septi-Chek AFB bottle**	**Isolator concentrate on Middlebrook 7H11 agar**
Mycobacterium tuberculosis	33	27	25	13
Mycobacterium simiae/SAV†	2	2	2	1
Mycobacterium bovis	1	0	1	0
Mycobacterium avium complex	1	1	1	1

* MFL: MYCO/F LYTIC bottle

† SAV: Mycobacterial species of the SAV group resemble *M. avium* complex by conventional biochemical tests, including a negative niacin reaction (true *M. simiae* have positive niacin reactions), but have high-performance liquid chromatography profiles that are consistent with *M. simiae*.

UTILITY OF NUMBER OF BLOOD CULTURE BOTTLES IN THE RECOVERY OF BLOODSTREAM PATHOGENS

Background. The rationale for inoculating two blood culture bottles with blood drawn either from a single venipuncture and divided, or from separate distinct blood draws, is based on the evidence that the likelihood of recovery of bloodstream pathogens is increased if larger volumes of blood are cultured [Hall et al., 1976; Ilstrup & Washington, 1983; Plorde et al., 1985; Tenney et al., 1982]. Weinstein and colleagues argued that, using conventional manual methods, a single set (i.e., one blood draw) of blood cultures will generally detect 91% of bacteraemia while two sets would detect >99% cases of bacteraemia [Weinstein et al., 1983].

The current studies in Tanzania, Thailand and Malawi have established that (i) in less-developed countries, where HIV-1 infection is prevalent, valid characterization of bloodstream infections in hospitalized adults necessitate culturing blood for mycobacteria, bacteria, and fungi; and (ii) culturing blood for the three groups of pathogens using conventional blood culture methods requires drawing up to 30 ml of blood from patients. However, limited blood culture supplies or microbiology capacity in less-developed countries often preclude obtaining >1 blood culture per patient, inoculating >1 bottle per blood draw, or culturing simultaneously for bacteria, mycobacteria, and fungi. Also, as mentioned earlier, patients from many of these countries do not like parting with the large volumes of blood that are necessary for culturing these three groups of pathogens.

The analysis in the previous chapter showed that 5ml of blood inoculated into one MFL blood culture bottle was a convenient method for culturing blood for bacteria, mycobacteria, and fungi and was as good as or superior to alternative, conventional blood culture systems. It

was suggested that the MFL bottle may be a useful tool for studying bloodstream infections in less-developed settings, where blood cultures already are part of a microbiology service repertoire or where existing resources limit blood culture service; or the MFL bottle may be used for conducting prevalence surveys of bloodstream infections.

Although inoculation of two MFL bottles has potential clinical benefit, it may be economically unsustainable if recommended for routine blood culture service in less-developed countries with limited resources. Therefore, further analyses of the Malawi data from the wet season were executed to determine the utility of inoculating blood from one draw consecutively into two MFL bottles for detection of bacteraemia, mycobacteraemia, and fungaemia in febrile, adult inpatients.

Results. During this study period, 228 study-patients had single blood draws inoculated into two MFL blood culture bottles; 56 (24.6%) of these study-patients had clinically significant bloodstream infections: 41 bacteria, 12 mycobacteria, and 3 fungi. *Salmonella* species and *M. tuberculosis* were the two most common isolates. Fifty-one (91%) of the 56 positive cultures were positive in both bottles. Of the 228 MFL pairs, 51 (22%) were both positive, 170 (75%) were both negative, and 5 (3%) had discordant results. Two (0.9%) of the 228 pairs of blood cultures yielded coagulase-negative *Staphylococcus* species that were considered skin contaminants and therefore were not included in the data analysis. The results are summarized in Table 18.

Table 18. Comparison of paired MYCO/F LYTIC (MFL) bottles for recovery of bacteria, mycobacteria, and fungi

Organism and result for first MFL bottle*	Result for second MFL bottle*	
	Positive	**Negative**
Bacteria		
Positive	38	3
Negative	0	170
Mycobacteria		
Positive	11	0
Negative	1	170
Fungi		
Positive	2	1
Negative	0	170

* The data represent numbers of bottles; none of the differences were statistically significant.

THE EMERGENCE OF *MYCOBACTERIUM SIMIAE* AND *SIMIAE-AVIUM* (SAV) GROUP BLOODSTREAM INFECTIONS IN PERSONS WITH THE ACQUIRED IMMUNODEFICIENCY SYNDROME—THAILAND AND MALAWI

Background. Persons with HIV-1 infection and AIDS are susceptible to disseminated mycobacterial infections involving blood, bone marrow, and multiple organs [Shafer et al., 1991; Grinsztejn et al., 1997]. In the United States, most such infections are caused by *Mycobacterium avium* or *M. intracellulare* (referred to collectively as *M. avium* complex). In less-developed nations where HIV-1 is endemic, *M. tuberculosis* is as or more prevalent than *Mycobacterium avium* complex [see previous chapters]. Accurate identification of mycobacterial species causing these infections is important for directing appropriate therapy, because different mycobacterial species are not uniformly susceptible to all antituberculosis agents.

Other mycobacterial species that have been reported to cause disseminated infection in HIV-infected persons include *M. simiae* and *simiae-avium* (SAV) group mycobacteria. SAV group organisms share characteristics of *M. avium* and *M. simiae* [Tortoli et al., 1997]. Disseminated infection with *M. simiae* has been reported in HIV-infected persons [Koeck et al., 1996; Levy-Frebault et al., 1987; Munier et al., 1993; Torres et al., 1991]. Within the SAV group, one distinct species, *M. triplex*, has recently been characterized [Floyd et al., 1996]. Subsequently, two case reports of disseminated infection caused by *M. triplex* have been reported: (i) an HIV-1-positive adult male patient in Italy [Cingolani et al., 2000] and (ii) a 13-year-old female patient who underwent a liver transplant in the United States [Hoff et al., 2001].

In the present study, four patients (two from Bangkok, Thailand and two from Lilongwe, Malawi) were found to have bloodstream infections caused by SAV group mycobacteria

Bangkok, Thailand

Patient 1, a 32 year-old-man, presented with fever, chronic cachexia, and diarrhoea of three month's duration. Physical examination revealed oral candidiasis and lymphadenopathy. The liver and spleen were not enlarged.

Patient 2, a 36-year old man, presented with fever, cachexia, and cough and shortness of breath of one week's duration. Physical examination revealed lymphadenopathy. The liver and spleen were not enlarged. Additional laboratory studies revealed marked anaemia and positive cerebrospinal fluid cryptococcal antigen.

Both Thailand patients were HIV-1-positive. Neither was receiving antiretroviral or antimycobacterial therapy at time of presentation. Both patients were treated with broad-spectrum antimicrobials for possible underlying bacterial infection and were discharged alive from the hospital.

Lilongwe, Malawi

Patient 3, a 28-year-old man, presented with chronic fever and cough of seven months duration. A physical exam revealed cachexia and skin lesions consistent with Kaposi's sarcoma. No lymphadenopathy or liver or spleen enlargement was noted.

Patient 4, a 36-year-old man, presented with fever, chronic cough and a five-month history of diarrhoea. Physical examination revealed oral candidiasis. There was no lymphadenopathy; liver and spleen were not enlarged.

Both Malawi patients were HIV-1-positive. Neither was receiving antiretroviral or antimycobacterial therapy at the time of presentation. Both were treated with penicillin and chloramphenicol for presumed underlying bacterial infection, and were discharged alive from the hospital.

Susceptibility testing of SAV Group mycobacteria. All four isolates were available for susceptibility testing. All were resistant to all first-line drugs used for treating *M. tuberculosis* infection (isoniazid, rifampicin, and pyrazinamide) and to alternative drugs used for treating multidrug-resistant tuberculosis (MDRTB).

CHAPTER 7

SUMMARY

DAR ES SALAAM, TANZANIA

This was a prospective study of 517 febrile (axillary temperature 37.5°C) adults (≥15 years) who were admitted consecutively to the largest teaching hospital in Tanzania. One hundred and forty-five (28 %) patients had bloodstream infections. Of these 145 patients, 118 (81 %) were HIV-1-infected. HIV-1-positive were more likely than HIV-1-negative patients to have bloodstream infection (118/282 vs. 27/235; P <0.0001). The three most frequently isolated pathogens were *M. tuberculosis* 60 (39 %), non-*typhi Salmonella* species 29 (19 %), and *Staphylococcus aureus* 13 (8.3 %). The malaria parasitaemia rate was similar to that of an afebrile control group of patients (9.4 % vs. 8 %). In this patient population with high prevalence of HIV-1 infection, *M. tuberculosis* has become the foremost cause of documented, community-acquired bloodstream infections.

BANGKOK, THAILAND

This was a study of 246 consecutive febrile patients admitted to a teaching hospital in Bangkok. One hundred and nineteen (48%) had bloodstream infections; 182 (74%) patients were HIV-1-infected. The two most common pathogens were *C. neoformans* (n=30) and *M. tuberculosis* (n=27). HIV-1-positive patients were more likely than HIV-negative patients to have mycobacteraemia (57/182 vs. 0/64; P <0.0001), fungaemia (38/182 vs. 2/64; P <0.001), or polymicrobial bloodstream infection (19/182 vs. 0/64; P <0.002). Mortality was higher among patients with bloodstream infection versus those without (P <0.001). Independent

clinical predictors for bloodstream infections included HIV infection, chronic diarrhoea, lymphadenopathy, or splenomegaly.

LILONGWE, MALAWI (DRY SEASON)

Of 233 patients who presented to a Malawi teaching hospital during the 1997 dry season, 173 (74%) were HIV-1-infected and 70 (30%) had bloodstream infections. The two most commonly isolated pathogens were *S. pneumoniae* (n= 25; 34 %) and *M. tuberculosis* (n= 21; 29 %). Nine (4 %) patients had malaria parasitaemia. Bloodstream infections were more likely in HIV-1 positive than HIV-1-negative patients (62/173 vs. 8/60; P <0.01). Independent clinical predictors of bloodstream infections included HIV-1 infection and altered mental status. Mortality was higher among patients with than in those without bloodstream infections (P <0.001).

LILONGWE, MALAWI (WET SEASON)

Of 238 patients presented to the same Malawi teaching hospital during the 1998 wet season, 173 (73%) were HIV-positive and 67 (28%) had bloodstream infection. The predominant wet season bloodstream infection pathogens were non-*typhi Salmonella* species (41%), *M. tuberculosis* (19%), and *Cryptococcus neoformans* (9%). (cf., the predominant dry season pathogen was *S. pneumoniae*). As predicted by earlier data, mycobacteraemia was more likely in HIV-positive than HIV-negative patients (13/173 vs. 0/65; P <0.05). A logistic regression model yielded clinical predictors of bloodstream infection that included chronic fever, oral candidiasis, or acute diarrhoea. Thus, the spectrum of pathogens causing bloodstream infection in febrile hospitalized patients may vary by season.

UNRECOGNISED (OCCULT) MYCOBACTERAEMIA

To determine whether *M. tuberculosis* bloodstream infections frequently go unrecognised, a comparative analysis of a subset of the Thailand and Malawi data was carried out. Of 344 patients, 255 (74%) were HIV-1-infected, median age was 33 (range 18-87) years, 208 (61%) were male. Thirty-four (10%) patients had *M. tuberculosis* bloodstream infection and 5 of these were already on antituberculosis therapy. Only HIV-1-infected patients had *M. tuberculosis* bloodstream infection. Of the 29 patients with *M. tuberculosis* bloodstream infection not already receiving antituberculosis therapy, 13 (45%) had an abnormal chest radiograph or a positive sputum smear. Sixteen (55%) patients had no additional diagnostic test results to indicate *M. tuberculosis* infection; 13 (81%) of these had cough. Thailand patients were more likely than Malawi patients to have chest radiographs or sputum smears performed (114/216 vs. 16/128; $P < 0.0001$); patients with *M. tuberculosis* mycobacteraemia in Thailand were more likely than those in Malawi to have received therapy or to have had additional diagnostic tests for the diagnosis of tuberculosis (14/20 vs. 4/14; $P < 0.05$). Thus, in regions of the less-developed world, where both *M. tuberculosis* and HIV infections are prevalent, disseminated *M. tuberculosis* infection may frequently go unrecognised.

MICROBIOLOGY—COMPARISON OF BLOOD CULTURE METHODS

During two study periods (study period I [1997] and study period II [1998]), blood from patients in Malawi was cultured using the *BACTEC*™ MYCO/F LYTIC (MFL), ISOLATOR™ 10 (Isolator), Septi-Chek™ AFB (SC-AFB), and Septi-Chek™ bacteria (SC-B) systems. During study period I, 251 blood cultures yielded 44 bacterial isolates. For bacteraemia, the MFL was similar to the Isolator concentrate on chocolate agar (34/44 vs.

27/44, P = NS), but more sensitive than the SC-B bottle (34/44 vs. 24/44, P = 0.05). For both study periods combined, 486 blood cultures yielded 37 mycobacterial and 13 fungal isolates. For mycobacteraemia, the MFL and Isolator concentrate in the SC-AFB bottle were similar (30/37 vs. 29/37, P = NS); the MFL bottle was more sensitive than the concentrate on Middlebrook agar (30/37 vs. 15/37, P = 0.002). For fungaemia, the MFL bottle was as sensitive as the SC-B bottle or Isolator concentrate on chocolate or IMA slants. Thus, the MFL bottle, inoculated with just 5 ml of blood and examined under ultraviolet light, provides a sensitive and uncomplicated method for comprehensive detection of bloodstream infections in less-developed countries.

MICROBIOLOGY—ONE VERSUS TWO BLOOD CULTURE BOTTLES FOR PATHOGEN RECOVERY

Inoculation of one bottle would be expected to reduce the sensitivity of MFL bloodstream pathogen detection. To ascertain the degree of this loss of sensitivity, blood was drawn from each of 228 febrile, adult inpatients in Malawi and 5 ml were inoculated into each of two MFL bottles. Of 228 paired bottles, 51 (22%) were both positive, 172 (75%) were both negative, and 5 (3%) were discordant. Eleven (92%) of 12 patients with mycobacteraemia and 38 (92%) of 41 patients with bacteraemia would have been detected had only one MFL bottle been inoculated. Thus, a second MFL bottle does not significantly increase diagnostic sensitivity.

NEW EMERGING BLOODSTREAM PATHOGENS—SAV GROUP MYCOBACTERIA

Four patients (two from the Thailand study and two from the Malawi study) were found to have bloodstream infections caused by the SAV Group mycobacteria. These mycobacteria have the biochemical profile of *M. avium* complex but the HPLC properties of *M. simiae*, and

are generally resistant to all first-line drugs used for treating *M. tuberculosis.* Patients with SAV mycobacterial infections may go unrecognised and presumed to be infected with multidrug-resistant *M. tuberculosis*.

CHAPTER 8

DISCUSSION

TANZANIA

The results of this study indicate that the prevalence of bloodstream infection among febrile adults admitted to the largest referral hospital in Tanzania was 28% and that *M. tuberculosis* complex was the most common cause of community-acquired bloodstream infection. Non-*typhi Salmonella* species were the second most frequent bloodstream pathogen, followed by *S. aureus, E. coli, S. pneumoniae,* and *C. neoformans*, respectively. Moreover, 55% of febrile adult admissions to this hospital were HIV-1-seropositive. The finding that *M. tuberculosis* was the most common bloodstream pathogen was unexpected, especially as previous studies in the Ivory coast, Rwanda, and Kenya had suggested otherwise, i.e., that non-*typhi Salmonella* species and *S. pneumoniae* were the predominant cause of bloodstream infection in sub-Saharan Africa [Gilks et al., 1990a Taelman et al., 1990; Vugia et al., 1993]. The heterogeneity of strains of *M. tuberculosis* demonstrated by RFLP suggests that the findings of this study were neither a result of an outbreak of *M. tuberculosis* bloodstream infection at MMC, nor due to inadvertent contamination of specimens in the microbiology laboratory.

A 1995 report from Nairobi had previously documented mycobacteraemia in 14 patients with advanced HIV infection. Of these 14 cases of mycobacteraemia, three (21 %) were due to *M. avium* complex (the first published report of disseminated *M. avium* complex infection in HIV-1-infected patients in sub-Saharan Africa); the other 11 (79 %) cases were caused by *M. tuberculosis* [Gilks et al., 1995]. The current study supports the finding that *M. avium* complex bloodstream infections are relatively uncommon in HIV-infected patients in Tanzania.

The SC-B bacteria biphasic blood culture system has been previously used in Africa to investigate non-mycobacterial bacteraemia [Vugia et al., 1993]. Although this system does not require specialized equipment, which would be impractical anyway in a country with limited resources, it is expensive relative to lysis-centrifugation. Mattar et al used lysis-centrifugation and inoculated concentrate onto various media selective for mycobacteria, including 7H9 broth, and demonstrated that the lysis-centrifugation technique is easy to perform and has a relatively high yield of AFB [Mattar et al., 1992].

The current study is the first in Africa in which the concentrate from lysis-centrifugation was inoculated directly into a biphasic bottle containing media selective for AFB, and confirms previous findings that liquid media enhance the recovery of small numbers of mycobacteria from clinical specimens [Sewell et al., 1993]. The SC-AFB bottle is a biphasic system that combines a paddle containing Middlebrook 7H11, modified egg-based, and chocolate solid media with a bottle containing 20 ml Middlebrook 7H9 broth and an internal CO_2 source. This mycobacteria culture system was chosen because it has been shown to have significantly greater sensitivity, compared with conventional solid media, for the recovery of all mycobacteria from clinical specimens [D'Amato et al., 1991; Giger et al., 1990].

Wasilauskas and Morrell demonstrated that the lysing anticoagulant in the Isolator tube inhibited the growth of *M. avium* complex [Wasilauskas and Morrell, 1994]; Doern and colleagues subsequently reported optimum recovery of *M. avium* complex from blood using a small volume of Isolator concentrate (0.2 ml) to inoculate the BACTEC 12B broth [Doern et al., 1994]. In the current study, 1 ml of concentrate was inoculated into the SC-AFB bottle. Although this likely optimized the overall recovery of mycobacteria, *M. avium* complex and *M. tuberculosis* yield might have been improved further by using a smaller Isolator

concentrate inoculation volume. A study comparing the yield of *M. tuberculosis* from blood cultures using large and small Isolator concentrate volumes inoculated into a mycobacterial biphasic system has not yet been done.

The overall rate of blood culture contamination in the current study was low (1.3%). True positive blood cultures were no more or less likely than negative cultures to have a contaminant. This was achieved by ensuring that high aseptic standards were maintained before and during venipuncture, using both alcohol and povodine iodine for skin cleansing and allowing the skin to dry properly before venesection, and by scrupulous cleaning of the rubber diaphragms of blood culture bottles and Isolator tubes with isopropyl alcohol before inoculation with blood or other sterile solutions during every stage of microbiologic processing and analysis. Three of the organisms considered contaminants were identified only on terminal subculturing of clear blood culture broth onto sheep blood plates, after seven days of incubation. Since these three organisms were most likely introduced into the inocula at the time of terminal subculturing and not at the initial venipuncture, the true contamination rate was almost certainly lower than the observed rate of 1.3%.

Without microbiology facilities and appropriate blood cultures, the prevalence of bloodstream infection in the febrile Tanzanian study population would not have been determined. While the prevalence of bloodstream infection has probably been underestimated in febrile adults, malaria parasitaemia may be overestimated as the underlying cause of fever. Tanzania already has an established mycobacteria reference laboratory. Thus, it might be more practical to develop existing blood culture facilities, particularly to detect mycobacteraemia and fungaemia. Surveillance in this area is vital especially as the current study has demonstrated that 95% of *M. tuberculosis* isolates remain sensitive to ethambutol,

isoniazid, rifampicin, and streptomycin.

Limited resources restrict the availability of antimicrobials in Tanzania and other sub-Saharan countries. Thus, identification of organisms isolated from blood cultures and antimicrobial susceptibility testing should promote appropriate antimicrobial prescribing. In the current study, 40% of *S. pneumoniae* isolates were resistant to penicillin and susceptibility testing suggested that chloramphenicol would have been the antimicrobial of choice for an infection caused by this pathogen. On the other hand, the number of bacterial isolates (including *S. pneumoniae*) tested for susceptibility to available antimicrobials were too small to enable specific conclusions regarding optimal choice of agents for specific infections in patients, or to enable reasonable estimation of the prevalence of antimicrobial-resistant organisms in the community. Susceptibility testing of isolates to panels of antimicrobials used for routine antimicrobial susceptibility testing in the United States were not carried out largely because many of these agents are not routinely available in most sub-Saharan countries, including Tanzania.

Although 37 (62%) of 60 patients with mycobacteraemia had pulmonary disease by history and physical examination, sputum smears for mycobacteria were available for only 10 (17%) of these individuals. Moreover, many patients did not obtain chest radiographs during their hospital stay for various reasons which included lack of clear clinical indication, inability of the patient to pay for a film, lack of trained hospital personnel, broken radiology machines, an overwhelmed radiology department, or death of the patient before a radiograph could be obtained. It is possible that many of the 23 patients with mycobacteraemia who exhibited no clinical evidence of pulmonary disease had occult pulmonary tuberculosis. However, because many of these patients did not have chest radiographs on admission for the various reasons

mentioned, the true number of patients with occult *M. tuberculosis* pulmonary or extra-pulmonary disease remains unknown. A mortality rate of 45% among study patients with mycobacteraemia suggests that further research needs to be conducted to determine the extent of occult *M. tuberculosis* disease in sub-Saharan Africa.

Based on the results of this study, the following were recommended: (i) establish basic facilities to rapidly identify AFB, common Gram-positive and -negative pathogens and their susceptibilities to available antimicrobials; (ii) expand microbiology capabilities to include lysis-centrifugation, which I demonstrated to be feasible and within the capabilities of the microbiologic staff at MMC; and (iii) encourage and train clinicians to actively seek mycobacterial infection in febrile patients clinically suspected to have HIV-1 infection. As has been the case in the United States for many years, it might be more appropriate to integrate mycobacteriology and bacteriology services rather than maintain them as separate entities as is the case in so many countries in Africa. This study suggests that providing basic microbiology laboratory facilities in sub-Saharan Africa will have significant impact on patient care.

The results from this Tanzania study suggest that (i) in less-developed countries with high prevalence rates of HIV infection, febrile adults should be evaluated for a wide spectrum of infectious agents, including mycobacteria, fungi, and bacteria; (ii) there is a need to expand the capability of laboratories in these countries to diagnose a wide range of emerging pathogens; and (iii) these data may help change policies for prophylactic or empirical antimicrobial therapy of febrile HIV-1-infected patients.

THAILAND

The data from the Thailand study phase indicate that the prevalence of bloodstream infection among febrile adults admitted to a large infectious diseases hospital in Bangkok, Thailand, was 48% and that *C. neoformans, M. tuberculosis,* and *M. avium* complex were the three pathogens most commonly isolated from the bloodstream of these patients. In addition, certain fungi (*H. capsulatum* and *P. marneffei*) and mycobacteria (*M. simiae*, *M. scrofulaceum*, and mycobacteria of the SAV group) emerged as important causes of bloodstream infection in this patient population. Three-quarters of adults with fever hospitalized at this Bangkok hospital and 96% of patients with bloodstream infection were HIV-1-seropositive. Multivariate analysis suggested specific clinical parameters that were strongly predictive for bacteraemia, mycobacteraemia, and fungaemia. Approximately one-quarter of *M. tuberculosis* isolates showed multidrug resistance.

Previously, the association between HIV-1 infection and tuberculosis had been described for HIV-1-endemic regions in Africa [Gilks et al., 1990b; Nunn et al., 1990; Nunn et al., 1992; De Cock et al., 1992] and in Brazil, where 19 (58%) of 33 HIV-1-infected patients with a final diagnosis of tuberculosis had *M. tuberculosis* bloodstream infection [Grinsztejn et al., 1997]. The data from the current Thailand study and from the previous Tanzania phase have now clearly established the predominance and importance of *M. tuberculosis* bloodstream infections in febrile HIV-1-infected adult patients in these countries. Thus, it is imperative that laboratories with adequate resources in areas of the world with high HIV-1 prevalence rates use methods that enhance recovery of *M. tuberculosis*, so these infections can be diagnosed early and appropriately.

The findings of the Tanzania phase of the current study and of work in Kenya [Gilks et

al., 1995] had suggested that while *M. tuberculosis* bloodstream infections are common in febrile adult HIV-1-infected patients, *M. avium* complex bloodstream infections remain relatively uncommon. The data from the current Thailand study suggest that, unlike these sub-Saharan studies, *C. neoformans*, *M. tuberculosis*, or *M. avium* complex bloodstream infections are relatively common in similar patient populations in Bangkok. That the prevalence rate of *M. avium* complex bloodstream infection in Bangkok is so different from the rates in similar patient populations in HIV-1-endemic regions in sub-Saharan Africa underscores the variation in the epidemiology of bloodstream infection in different countries and the importance of capacity building so that regional reference laboratories can culture blood (and other specimens) comprehensively for bacteria, mycobacteria, and fungi. In addition, these results demonstrate the inherent risk of extrapolating data from one HIV-1-endemic region of the world to another for the implementation of clinical or public health policies.

The multivariate analyses provide a statistical model that highlights which febrile hospitalized adults in a large Bangkok hospital are at greatest risk of bloodstream infection and should be strongly considered for blood culture or empiric antimicrobial therapy. The results suggest that (i) any adult HIV-1-infected patient presenting with chronic diarrhoea, chills and rigors, and an absolute lymphocyte count under 1200/mm^3 would have >90% probability of having a bloodstream infection; (ii) any adult patient with fever and a history of chronic diarrhoea should be considered for bacterial blood cultures, at the very least; and (iii) patients with lymphadenopathy, an abnormal white blood cell count, and clinically suspected to be HIV1-infected, should be considered for further microbiological investigation, where appropriate, and if comprehensive laboratory resources and services are available. If blood culture services are unavailable, empirical therapy alone may be appropriately based on these

probabilities. Development and use of such algorithms, after discussion between microbiologists, clinicians, and epidemiologists, might facilitate better use of scarce laboratory resources and improve patient outcome.

In some countries or regions, empirical antimicrobial therapeutic decisions may be based on limited laboratory results. Importantly, bloodstream infection prevalence rates vary by country, and clinical predictors for bloodstream infection might vary from setting to setting. This precludes direct extrapolation of data from one country to another, and clinical algorithms developed for one specific site or country might not be universally applicable to other sites. Hence, countries that wish to implement public health policy should conduct appropriate bloodstream infection studies within their own setting or exercise care when extrapolating data from other sources. Cohort-based microbiologic studies, such as the present study, could guide appropriate empirical antimicrobial therapy.

RFLP analysis of the *M. tuberculosis* isolates in the current Thailand study demonstrated a heterogeneity of strains suggesting that the findings were neither a result of an outbreak of *M. tuberculosis* infection among hospitalized patients, nor a result of inadvertent contamination in the microbiology laboratory. As for Tanzania, a decision to treat coagulase-negative staphylococcus as a contaminant was based on the results of studies that demonstrated the clinical non-significance of these organisms as causes of bloodstream infections in the absence of invasive devices [Weinstein et al., 1997]. None of the patients enrolled in the current study had *in situ* intravascular devices at the time blood was drawn for culture. Thus, even if a substantial number of blood cultures had yielded growth of coagulase-negative staphylococcus, their clinical significance as true bloodstream pathogens would still have remained doubtful. The current findings also demonstrate that when blood cultures are

obtained by trained personnel using appropriate skin preparation, and specimens are handled appropriately in the laboratory, contamination is a relatively infrequent event (i.e., <1 %).

In Thailand, all *S. typhimurium* isolates were resistant to ampicillin and TMP/SMZ and 22% of *M. tuberculosis* isolates showed multidrug resistance. These results demonstrate the value of antimicrobial susceptibility information in the clinical selection of antimicrobial therapeutic regimens and underscore the importance of surveillance of antimicrobial resistance among pathogens that commonly cause bloodstream infection in HIV-1-endemic less-developed countries.

C. neoformans was the most common pathogen causing bloodstream infections in the Thailand study population; four patients had bloodstream infection due to *P. marneffei*, which is one of the most common pathogens causing opportunistic infections in HIV-1-infected patients in northern Thailand [Vanittanakom et al., 1997; Chariyalertsak et al., 1996]. The prevalence of *P. marneffei* infections among HIV-1-infected patients from areas outside the northern regions appears to be considerably lower [Chariyalertsak et al., 1996], and may be due to the fact that the HIV-1 epidemic has been most severe in the northern provinces of Thailand [Weniger et al., 1991].

In a study spanning a 4-year period, Chariyalertsak et al demonstrated that HIV-1-infected patients presenting with *P. marneffei* infection were more likely to be seen in the rainy season in each year; these investigators did not observe seasonal variation in the incidence of disseminated *C. neoformans* infections [Chariyalertsak et al., 1996]. The current study was conducted during the height of the Thailand dry season. Thus, the relatively low incidence of *P. marneffei* bloodstream infections in the Bangkok study patient population could have been a manifestation of seasonal variation in the occurrence of *P. marneffei*

infections.

The mortality rate among study-patients with bloodstream infection was significantly higher than that of patients without bloodstream infection (mortality was particularly high among patients with bacteraemia or fungaemia versus those with mycobacteraemia). The blood culture system in the clinical microbiology laboratory of the study hospital had the capability of detecting Gram-positive or -negative bacteraemia, but was less sensitive for recovery of mycobacteria or fungi. Without comprehensive blood cultures, bloodstream infections in the patients that I studied would have gone undetected. The findings in the Thailand component suggest that the initial priority of other Thailand hospitals ought to be towards the development of microbiology services to diagnose bloodstream infection, and identification of the patient populations most at risk of having bloodstream infection through ascertainment of clinical predictors. By focussing available resources on obtaining blood cultures from high-risk patient populations using comprehensive methods to detect bacteria, mycobacteria, and fungi, clinicians could initiate directed (versus blind) empirical therapy later tailored to the results of cultures.

In many countries, limited resources may result in patients having no microbiology tests performed; or, where available, use of inadequate methods that include use of expired or inappropriate culture media or for optimal recovery of organisms, varying duration of incubation, or omission of essential quality control methods. The Western style inpatient-based culture methods cannot be established or sustained in such resource-poor settings. Cohort-based microbiologic surveillance studies focus on quality-controlled diagnostic testing over a finite period and are performed on patients with similar symptoms or signs, such as fever. Such studies could be conducted at sentinel hospitals that provide a natural gathering

point to sample patients meeting these simple, objective entry criteria.

These cohort-based studies could be conducted in the form of periodic surveys of cohorts of patients meeting the aforementioned defined entry criteria using quality-controlled tests with a high positive predictive value for infection, such as cultures of blood, cerebrospinal fluid, or stool. A comprehensive cohort-based study conducted over a finite period may be more feasible and effective than individual patient-directed laboratory testing in providing useful clinical and public health information. Such studies may provide better estimates of true incidence and prevalence rates of emerging pathogens and antimicrobial resistance, and better determination of clinical predictors of infection. In addition, these studies would provide the opportunity to build capacity in basic clinical microbiology in sentinel regional hospitals or laboratories where there is inadequate critical public health infrastructure, or where full scale microbiologic services are unsustainable.

The results of the current study suggest that the HIV-1 pandemic has led to high rates of presentation of febrile adult patients with bloodstream infections to a large infectious disease hospital in Southeast Asia. The scope of clinical microbiology services offered by hospitals serving HIV-endemic areas in Thailand may need to be widened to include blood cultures for pyogenic bacteria, mycobacteria, and fungi. Development of algorithms based on clinical predictors of bloodstream infections may be a useful approach to more effectively use limited resources and improve patient care. Cohort-based microbiologic studies used for surveillance of emerging infections in sentinel hospitals may provide important clinical and public health information, such as clinical predictors of infection, identities of emerging pathogens and their antimicrobial susceptibility profiles of to commonly used antimicrobials, and prevalence rates of infections caused by these pathogens.

The daunting task remains to define the role of new and emerging pathogens causing bloodstream infection in a variety of patient populations presenting to hospitals in Southeast Asia and other less-developed countries. Continued recommendation of individual patient-directed culturing in less-developed countries is likely to fail because of insufficient laboratory personnel, resources, and expertise. The cohort-based methods used in the current study could be implemented at regional referral laboratories in less-developed countries. In this way, larger numbers of patients could be cultured using quality controlled methods. With the demonstrated utility of cohort-based microbiologic studies for the surveillance of emerging infections, there is hope that patient therapy can be improved and national and international surveillance of new and emerging pathogens and antimicrobial resistance can be enhanced.

MALAWI (DRY SEASON)

The bloodstream infection prevalence rate in adult patients presenting with fever to a large general hospital in Central Malawi was 30%; the HIV-1 seroprevalence and malaria parasitaemia rates in this study cohort were 74% and 4%, respectively. The rates of positive blood cultures increased as the number of clinical predictors present increased, ranging from 11% for patients with none of the independent clinical predictors of bloodstream infection to 100% in patients with all three independent risk factors. The rate of bloodstream infection in the study population in Malawi was higher than the rates found in Nairobi (10%), Abidjan (17%), or Kampala (24 %) [Gilks et al., 1990a; Vugia et al., 1993; Ssali et al., 1998]. Although these differences in the prevalence of bloodstream infection may reflect different rates of HIV-1 infection or differences in the stage of HIV-1 infection when patients present to the hospital, they also reflect the sensitivity of various blood culture methods used in the Nairobi, Abidjan, and Kampala studies. In contrast, the Tanzania phase of the current study employed blood culture methods similar to those used in Malawi phase and an identical patient case definition (fever) for enrollment, and documented a bloodstream infection prevalence rate that was similar to that documented during the Malawi dry season (28% vs. 30%).

In the present study, *S. pneumoniae* was the most common pyogenic bloodstream pathogen. This finding, though similar to that in the Kenya and Uganda studies [Gilks et al., 1990a; Ssali et al., 1998], contrasts with the findings in Tanzania, where culture techniques were similar to those used in Malawi, and with the findings in Cote d'Ivoire [Vugia et al., 1993]. Although differences in the prevalence rates of *S. pneumoniae* may reflect differences in culture technique, other possible explanations for these differences in the rate of *S. pneumoniae* infection include geographic location (both Dar es Salaam and Abidjan are closer

to seacoasts than is Nairobi or Lilongwe), seasonal variation, or exposure to antimicrobials, such as ampicillin. The prevalence rate of HIV-1 infection in the Malawi dry season study population is high and reflects several factors and potential biases, including the selection criteria used to enroll patients, socioeconomic factors in the population served by the hospital, and the dynamics of the HIV-1 epidemic in various regions of sub-Saharan Africa. The HIV-1 infection rate (74%) in the Malawi patients was similar to that found in Uganda in 1998 [Ssali et al., 1998], but was higher than reported rates in Kenya (1989), Cote d'Ivoire (1991), and Tanzania (see Tanzania results in chapter 5). These differences in HIV-1 prevalence may relate to geographic location, year of the study, or unchecked progression of the HIV epidemic.

The relatively low prevalence rate (4%) of malaria parasitaemia in the Malawi dry season study cohort was not significantly different from the rate (7%) in a control group of healthy afebrile adults. Given that 49% of the patients in the current study were initially diagnosed with malaria and the common perception among the lay public and healthcare workers alike that malaria is a common cause of fever, one may conclude that during the dry season, adult patients presenting to LCH with fever were frequently over-diagnosed with malaria. It is possible, however, that study patients with negative malaria smears were already on antimalarial prophylaxis or therapy that could have been obtained from community clinics or bought over-the-counter and administered before presenting to LCH.

As was shown in Tanzania, the data from Malawi confirm that *M. tuberculosis* is a frequent cause of bloodstream infection in sub-Saharan African countries while *M. avium* complex is an uncommon cause of disseminated infection among HIV-1-infected patients. These findings are compatible with the results of other studies in sub-Saharan Africa [Gilks et

al., 1995; Morrissey et al., 1992; Okello et al., 1990; Ssali et al., 1998; von Reyn et al., 1996]. In addition, some of these other researchers had come to the conclusion that disseminated *M. avium* complex infection in HIV-infected patients was higher in developed than less-developed countries and was probably related to both differences in exposure and differences in immunity [von Reyn et al., 1996]. Only one patient in the current Malawi study had *M. avium* complex bloodstream infection, the fifth reported case of disseminated *M. avium* complex infection in an HIV-1-infected patient in sub-Saharan Africa (the previous cases were documented in the current Tanzania study [see results section] and Nairobi [Gilks et al., 1995].

The relative paucity of disseminated *M. avium* complex bloodstream infection is perplexing given the ubiquitous presence of *M. avium* complex in the environment of sub-Saharan Africa [Morrissey et al., 1992; von Reyn et al., 1993]. However, von Reyn and colleagues also found that isolation rates of *M. avium* complex in water supply systems were higher in the United States and Finland than they were in Kenya and Zaire (32% vs. 5%; P= 0.056), and that none of the water supply samples from hospitals in Kenya and Zaire tested positive for *M. avium* complex, while about 20% in the United States and 50% in Finland did von Reyn et al., 1993]. Thus, the most plausible explanation for the low occurrence of *M. avium* complex infection in sub-Saharan Africa might lie in the fact that *M. avium* complex infection is a condition associated with piped water, of which there is relatively less in Africa compared with developed countries in the Northern Hemisphere.

The findings in the current Malawi study indicate that HIV-1 infection is an independent risk factor for bloodstream infection in newly hospitalized, febrile adults. Therefore, knowledge of HIV-1 status in these patients should facilitate the decision to initiate empirical

antimicrobial therapy. However, one cannot make a blanket recommendation that HIV-1 testing should be done routinely in areas of high HIV-1 seroprevalence in order to make a decision about initial empirical therapy because many hospitals in Africa do not have the necessary financial or laboratory resources to test every patient suspected of having HIV-1 infection. Even in institutions where HIV-1 testing services are available (e.g., LCH), it may take up to 5 days before test results become available to clinicians. Thus, HIV-1 serology test results cannot realistically be used to make clinical decisions for newly hospitalized febrile patients in HIV-1-endemic areas in less-developed countries. Notwithstanding these limitations, Malawian physicians have a low threshold of suspicion for HIV-1 infection and generally are able to suspect underlying HIV-1 infection from the history and physical examination alone.

The current Malawi data suggest that febrile patients with suspected or documented HIV-1 infection and clinical features, such as a history of chronic symptoms (fever, diarrhoea, weight loss, or cough), altered mental status, herpes zoster scars, pulmonary physical signs, or oral candidiasis should be suspected of having bloodstream infection (bacteraemia, mycobacteraemia, or fungaemia). The physician might wish to consider ancillary diagnostic tests, such as chest radiographs and/or sputum smear microscopy for bacteria and AFB, referral to the tuberculosis clinic, where appropriate, or initiation of empirical antibacterial or antituberculosis therapy. Febrile patients with signs of pulmonary consolidation alone are at increased risk of having a bacteraemia, probably caused by *S. pneumoniae,* and should be considered for bacterial blood cultures, if available, or treated with an appropriate empirical regimen. Since *S. pneumoniae* appears to be the most common bloodstream pathogen during the Malawi dry season, the spectrum of the empirical therapeutic regimen should be effective

against this organism. The *S. pneumoniae* isolates were largely resistant to TMP/SMX and penicillin but susceptible to tetracycline, erythromycin, and chloramphenicol. *Salmonella* species were generally resistant to TMP/SMX and gentamicin but were susceptible to chloramphenicol. Thus, chloramphenicol, depending on availability, should be considered for empiric therapy in patients suspected of having a bloodstream infection.

The current data also suggest that during the dry season, newly admitted, febrile, adult patients with negative malaria blood smears are more likely to have bloodstream infection; patients with altered mental status are more likely to have bloodstream infections than cerebral malaria. Since patients frequently attend their local clinic or district community health centre for antimalarial treatment at the onset of fever or other malarial symptoms, those with a definite diagnosis of malaria would have responded to this therapy and therefore have no need to attend LCH. In contrast, patients who did not respond to initial antimalarial therapy would have been more likely to have an infection other than malaria and therefore would have been the ones most likely to present to LCH. This may explain why patients with negative blood smears were more likely to have a bloodstream infection. Studies, such as this, contribute to the global surveillance of emerging infectious diseases and antimicrobial resistance. *M. simiae* and possibly mycobacteria of the SAV-group represent emerging infections in HIV-1-infected patients of the developed world [Huminer et al., 1993; Wald et al., 1992; Floyd et al., 1996]. The finding of these agents in the bloodstream of patients who participated in the Thailand study, and now in Malawi, suggest the emergence of these pathogens in the less-developed world may be related to the HIV epidemic.

Documentation of decreased susceptibility to penicillin among *S. pneumoniae,* and to TMP/SMX and gentamicin among *Salmonella* species isolated from the bloodstream suggest

that emerging antimicrobial resistance may be an increasing problem in sub-Saharan Africa. Followup studies may further characterize the diversity of infecting bloodstream pathogens and their clinical predictors; these data can be used to better target limited antimicrobials and improve patient outcomes in settings with limited resources.

MALAWI (WET SEASON)

Whoever wishes to investigate medicine properly, should proceed thus: in the first place to consider the seasons of the year, and what effects each of them produces.

Hippocrates (460? – 377 BC)

Despite this ancient admonition, little has been published about the seasonality of bloodstream infections in developed countries [Flournoy et al., 1983]. There is also a paucity of published data on the role of seasons in the occurrence of bacteraemia in less-developed countries where resources for diagnosis and treatment of life-threatening infections are limited. Mabey et al. were among the first to demonstrate seasonal variation in the occurrence of *Salmonella* septicaemia among paediatric patients in The Gambia [Mabey et al., 1987]. Data from published studies of bloodstream infection in hospitalized adults in various sub-Saharan African countries [Gilks et al., 1990; Vugia et al., 1993; Batchelor et al, 1996; Ssali et al., 1998] and from the current studies in Tanzania, Thailand, and Malawi have demonstrated varying distributions in bloodstream infection pathogens. Moreover, as was documented in the introductory chapter of this thesis, most of the published studies of adult populations [Gilks et al., 1990; Vugia et al., 1993; Batchelor et al, 1996; Ssali et al., 1998] utilized limited laboratory methods, were conducted during one seasonal cycle, or included cumulative annual data that did not permit investigators to determine whether the distribution of bloodstream infection pathogens varied by season. In the previous chapter, it was established that the predominant pathogens causing bloodstream infection during the Malawi dry season are *S. pneumoniae* and *M. tuberculosis*.

The results from the wet season phase in Malawi confirmed that the overall bloodstream

infection rate in a febrile, adult population hospitalized at the same Malawi District General Hospital during the 1998 rainy season was 28% and that the three most commonly isolated pathogens were non-*typhi Salmonella* species (41%), *M. tuberculosis* (19%), and *C. neoformans* (9%). The HIV-1 seroprevalence rate in the study population was high (73%), and the rates of pyogenic bacteraemia, mycobacteraemia, and fungaemia increased as the number of specific clinical predictors for each of these types of bloodstream infection increased. The previous study of a similar adult inpatient population at the same hospital during the 1997 dry season used the same case definition (fever), microbiologic methods, and blood culture inoculating volume, and showed similar bloodstream infection (28%) and HIV-1 seroprevalence (76%) rates. In contrast, the bloodstream pathogens most commonly isolated in the dry season study were *S. pneumoniae* (33%) and *M. tuberculosis* (26%).

Although seasonal variations in disease occurrence, especially for various infectious diseases, have been established by epidemiologists and clinicians over the years, very little data have been documented for seasonal variation in the incidence of bloodstream infections. In 1983, Flournoy and colleagues observed a seasonal pattern for *S. pneumoniae* bloodstream infection in a United States institution---a monophasic pattern that peaked from December to April [Flournoy et al., 1983]. In a study of bloodstream infections in Gambian children during 1979-1984, Mabey et al. found that the incidence of *Salmonella* septicaemia was more closely related to changes in transmission of malaria than to changes in the stool carriage rate of salmonellae, and that 74% of cases of non-*typhi Salmonella* septicaemia occurred during the rainy season in The Gambia [Mabey et al., 1987]. More recently, other researchers in Malawi established a seasonal pattern for bloodstream pathogens in febrile adult medical admissions to the largest hospital in the country: *S. pneumoniae* bacteraemia was more common in the cold

dry months while non-*typhi Salmonella* spp. predominated following a rise in temperature [Gordon et al., 2001]. Suggested reasons for seasonal occurrence of bloodstream infections include variation in virulence of bacterial pathogens, host susceptibility, patient demographics and behaviour, environmental factors, such as weather, or occurrence of underlying diseases, such as HIV-1 infection [Flournoy et al., 1983; Mabey et al., 1987]. Mabey et al. suggested an explanation for the increased incidence rate of non-*typhi Salmonella* spp. in the hot months: i.e., because raised levels of immune complexes persist in the serum for several weeks after an acute attack of *P. falciparum* malaria, the immune complexes might cause impairment of macrophage function throughout this phase and may render the patient susceptible to invasive *Salmonella* infections [Mabey et al., 1987].

Previous studies of bloodstream infections in sub-Saharan Africa either were conducted during one particular season, or combined data over seasonal cycles [Alausa et al., 1977; Batchelor et al., 1996; Gilks et al., 1990a; Ssali et al., 1998; Vugia et al., 1993]. The importance of season-specific surveillance is clearly illustrated by the different recovery rates despite similar culture methods for non-*typhi Salmonella* and *S. pneumoniae* in the wet and dry seasons. In more affluent settings, initial empirical therapy would be initiated to treat a broad spectrum of potential pathogens. Then, laboratory tests would be applied to focus treatment on a specific pathogen. In many less-developed countries, confirmatory laboratory tests are not available or the microbiologic methods (e.g., type of media available, duration of incubation, requirements for quality control) may be inadequate. Where cost or availability prohibits the use of multiple antimicrobials, the clinician often is compelled to choose one empiric regimen. Thus, the patient's survival may depend on the clinician's awareness of the distribution of bloodstream infection pathogens that he is potentially dealing with.

To improve patient outcomes and enhance rational use of antimicrobials, hospitals in less-developed countries with limited laboratory resources may need to improve the accuracy of probable diagnoses and choice of antimicrobials used for initial empiric therapy. Establishing comprehensive microbiology laboratories at all hospitals in these settings may be inappropriate or unrealistic. A better approach would be identification of regional or sentinel microbiology laboratories and utilization of resources in these laboratories to conduct cohort-based studies. Such studies are conducted as periodic surveys of patients who meet simple, objective entry criteria, such as symptoms, signs (e.g., fever, diarrhoea, rashes, or altered mental status), or syndromes. Cohort-based microbiologic sample surveillance studies, such as these series of blood culture surveys, focus on quality-controlled diagnostic testing with a relatively high positive predictive value for infection, such as cultures of blood, cerebrospinal fluid, or stool, and may be more feasible and effective than continuous, individual, patient-directed laboratory testing in providing useful clinical information. In this way, the distribution of pathogens associated with various infections, and their susceptibilities to available antimicrobials would be determined. These data could then be used to facilitate selection of the most appropriate agents for empirical therapy between study periods. In addition, these studies provide the opportunity to enhance basic clinical microbiology capacity in sentinel regional hospitals or laboratories in need of critical public health infrastructure. In the current study, available resources were focussed on obtaining blood cultures in a high-risk patient population using comprehensive methods to detect bacteria, mycobacteria, and fungi onsite. These methods include relatively simple but highly efficient blood culture techniques that can be effectively applied even in an underdeveloped setting.

Another cost-effective approach to the use of limited microbiology resources would be to

obtain blood cultures or initiate empirical therapy on clinical predictors or syndromes. The data from the present study suggest that although the predictive value positive of clinical correlates of bacteraemia, mycobacteraemia, or fungaemia are limited in scope, they are potentially useful for the development of therapeutic or diagnostic algorithms. For example, patients presenting with acute diarrhoea, vomiting, jaundice and a high fever may be considered to be at increased risk of acquiring a bloodstream infection, probably caused by non-*typhi Salmonella* species, and ought to be considered for bacterial blood cultures, where these services are available, or treated with an appropriate empirical regimen. Febrile, adult patients with a history of chronic cough and a family history of cough may be considered at risk of acquiring disseminated *M. tuberculosis* and should be examined with a chest radiograph and sputum smear at the very least.

Although differences in the prevalence of bloodstream infection may reflect different rates of HIV-1 infection or differences in the stage of HIV-1 infection when patients present to the hospital, they also reflect the sensitivity of various blood culture methods used in these studies, especially for isolation of mycobacteria. Although the contribution of mycobacteraemia to acute morbidity may be small, it may be necessary to assess whether the relatively large number of patients with *M. tuberculosis* bloodstream infection poses a significant infectious risk to others in the hospital and in their communities.

The issue of hospitalized patients who do not have symptoms or signs of *M. tuberculosis* infection but have disseminated tuberculosis as evidenced by mycobacteraemia (occult mycobacteraemia) at the time of admission was observed in the first phase of the study in Tanzania, but requires further study. Identification of clinical predictors of *M. tuberculosis* bloodstream infection may provide an early opportunity to determine which patients have a

high probability of disseminated tuberculosis and therefore should be screened early on with chest radiographs and sputum smears.

Febrile illnesses are responsible for a significant number of hospital admissions in the countries where the current series of studies were executed, and in other parts of sub-Saharan Africa [Ngaly and Ryder, 1988]. Moreover, such febrile illnesses often are attributed to malaria [Nwanyanwu et al., 1997]. Although the rate of malaria parasitaemia was significantly higher during the wet season study-patients compared to those in the dry season study-cohort (31% vs. 4%; $P < 0.001$), clinical malaria was markedly overdiagnosed in both seasons. In the wet season, 56% of the study-patients diagnosed with malaria were incorrectly diagnosed. Of those patients incorrectly diagnosed and treated for malaria, 34% had bloodstream infections caused almost exclusively by Gram-negative pathogens.

Although malaria is an illness of well-known seasonality, treatment algorithms often fail to reflect this [Aniedu, 1997; Hay et al., 1998]. Resources may be unnecessarily spent on providing empiric therapy at constant levels throughout the year for an infection whose etiologic agent varies seasonally. Taking seasonal variations in disease prevalence into consideration could strengthen therapeutic algorithms for hospitalized patients with fever.

In developed countries, confirmation of HIV-1 serologic tests with Western blot molecular technique is standard in many laboratories. In contrast, there is continuing debate about the usefulness of expensive HIV-1 confirmatory tests in less-developed countries where the Western blot often is not used because of its complexity and high cost. In hospitals in sub-Saharan Africa and Southeast Asia, where 50-75% of adults who present to hospitals with fever may be HIV-1-infected [Batchelor et al., 1996; Gilks et al., 1990a; Ssali et al., 1998], it may not be economically feasible to conduct HIV-1 confirmatory testing, though a number of

false HIV-positive test results would, of course, be expected. Ittiravivongs et al in Thailand, and Urassa et al in Tanzania showed that use of two ELISA tests to confirm the presence of HIV-1 antibodies produces results comparable to those of the Western blot [Ittiravivongs et al., 1996; Urassa et al., 1992]. In the current series of studies in Tanzania, Thailand, and Malawi, there was 100% concordance between the final results of two ELISA antibody tests and Western blot..

Variation in the data from the studies show that results cannot be extrapolated to other regions or countries. Rather, the approach of regional, season-specific, surveillance studies is offered as a model for optimizing patient care where routine laboratory testing is not available. Selective application of diagnostic laboratory resources can provide answers to clinical questions that can be generalized within a region, greatly extending the effects of limited resources, improving patient care, and enhancing the recognition of emerging pathogens or antimicrobial resistance.

UNRECOGNISED (OCCULT) MYCOBACTERAEMIA

Much current emphasis on controlling the global increase in tuberculosis is placed on promoting directly observed treatment, short course (DOTS). The main objective of this prevention effort, led by the World Health Organization [World Health Organization, 1998], is to achieve increased cure rates through greater compliance with antimicrobial therapy. This emphasis is appropriate given that both HIV-1-infected and noninfected patients may be rendered non-infectious for tuberculosis through treatment, and that the emergence of multidrug resistance in *M. tuberculosis* is mainly a result of non-compliance with therapy.

Primary prevention of *M. tuberculosis* infection, however, is another important part of controlling the worldwide increase in tuberculosis. The hospital setting is an epicentre for the spread of tuberculosis particularly in less-developed countries where *M. tuberculosis* and HIV infection are prevalent. In such healthcare settings, highly susceptible patients, many with advanced HIV infection, are in close proximity to others with active tuberculosis. Although transmission of *M. tuberculosis* in such settings may appear inevitable, even simple infection control efforts, such as early diagnosis and treatment, or the isolation of groups of patients with active disease, may have a major impact on nosocomial transmission rates.

The current studies show that about 10% of patients with fever, admitted to an infectious disease hospital in Thailand and a general hospital in Malawi had bloodstream infections caused by *M. tuberculosis*; 30% of patients in the infectious disease hospital and 71% of patients in the general hospital were not thought to have active tuberculosis disease because they had not received previous antituberculosis therapy, chest radiography, or sputum smear results at the time of admission. The greater recognition of active disease in the infectious disease hospital was associated with greater overall availability or use of antituberculosis

therapy, chest radiographs, and sputum smears. The greater availability or use of antituberculosis drugs at the infectious disease hospital was associated with higher rates of drug resistance among *M. tuberculosis* isolates.

The failure to identify *M. tuberculosis* in blood often is in many cases the result of use of inappropriate culture media, insufficient incubation of cultures, or failure to obtain blood cultures for mycobacteria. Important for the execution of this study was the availability of simple and effective means to isolate mycobacteria from blood [Reimer, 1994]. Although the current study incorporated a commercial system to lyse white blood cells in blood culture specimens before inoculating onto commercial liquid and solid media, noncommercial systems have been devised for the effective recovery of mycobacteria from the blood [Fandinho et al., 1997]. Inoculation of the lysis-centrifugation pellet into liquid or biphasic media may result in inhibition of mycobacterial growth [Wasilauskas & Morrell, 1994]. This inhibition was overcome by inoculating only 0.5 ml of the pellet into the biphasic media broth [Doern & Westerling, 1994] and inoculating part of the pellet directly onto Middlebrook 7H11 agar slants.

HIV-1-infected patients are more likely to have extrapulmonary disease than are HIV-1-negative individuals; chest radiographs were not done routinely in the study populations in Malawi. Therefore, it was not possible to ascertain with certainty whether all patients with *M. tuberculosis* bloodstream infection had pulmonary involvement. However, in a previous study of HIV-1-infected patients selected on the basis of having extrapulmonary disease, many were found to have viable *M. tuberculosis* in their sputum [Shafer et al., 1991]. In other studies, lung involvement was found in most patients with *M. tuberculosis* bacteraemia, who were assessed with chest radiographs [Barber et al., 1990; Bouza et al., 1993; Grinsztejn, 1997]. In

addition, the fact that most patients in the current study with unrecognised tuberculosis infection had symptoms of acute or chronic cough is of concern. Even if the bacterial load in the sputum of such patients is low at the time of admission, one must expect increased infectivity while the patient is in hospital unless active disease is recognised and therapy promptly initiated.

The proportion of all infectious patients (i.e., those admitted to the hospital with active tuberculosis) detected by these surveys is unclear. In retrospective studies of HIV-1-infected and non-infected patients with active tuberculosis only 14% -15% of those tested had mycobacteraemia [Bouza et al., 1993; Shafer et al., 1989]. However, in a prospective survey of 33 HIV-1-infected patients with active tuberculosis that used the lysis centrifugation technique for blood culture, Grinsztejn and colleagues found mycobacteraemia in 60% of patients; in five (15%) patients, blood was the only specimen that yielded *M. tuberculosis* [Grinsztejn et al., 1997]. In another study of 11 AIDS patients with pulmonary tuberculosis, all had mycobacteraemia by the lysis-centrifugation technique [Mattar et al., 1992]. Although as many as one-third of patients who present to hospitals with active tuberculosis may be afebrile, the proportion is probably much smaller among HIV-1-infected patients [Shafer et al., 1991]. Therefore, although the present study studies were limited to a blood culture evaluation of febrile patients, available data suggest that most patients with advanced HIV-1 infection and active tuberculosis were ascertained [Shafer et al., 1989; Mattar et al., 1992; Nunn et al., 1992].

Patients with unrecognised active tuberculosis were defined on the basis of the absence of previous therapy or abnormal chest radiograph, or positive AFB sputum smear results from a test carried out on or before the day of admission. The under-recognition of active disease

was associated with lower rates of previous therapy and diagnostic testing. Although some clinicians advocate analysis of medical records as a means of assessing whether active tuberculosis is present in patients, the criteria used to define these patients in the present blood culture surveys offer more objective evidence as to whether tuberculosis was seriously suspected by the admitting clinicians. Evaluation of an old test, the chest radiograph, supports its role in the diagnosis of tuberculosis [Barnes et al., 1988]. AFB sputum smears have an established role in diagnosing active disease [Kramer et al., 1990; Modilevsky et al., 1989; World Health Organization, 1998] and are important for determining infectivity for infection control purposes [Centres for Disease Control and Prevention, 1994]. Traditionally, the diagnosis of active tuberculosis could be achieved with a fair degree of accuracy without the aid of additional diagnostic tests. In the HIV era, however, this is no longer true [Elliott et al., 1993; Gilks et al., 1990b; Kramer et al., 1990; Modilevsky et al., 1989; Shafer et al., 1991].

The challenges faced in the global battle against tuberculosis are tremendous. Results from this study emphasize these challenges and the overwhelming nature of the problem. One encouraging finding, however, is the association of a lower rate of antimicrobial resistance with the under-recognition of active disease.

Nonetheless, global tuberculosis-control efforts should also focus on primary prevention by improving the availability and use of chest radiographs and sputum smears to diagnose active disease. The problem is that when chest radiographs have not been obtained, one does not know if they would have been abnormal. Thus, initiating empirical therapy in patients with suspected active tuberculosis is more important. The findings of the present study suggest that these tests should be carried out at hospitals in less-developed countries on all febrile adult patients with oral thrush, chronic cough, fever, or weight loss. In less-developed

countries even with clinical microbiology facilities, testing every febrile patient admitted to hospital for mycobacteraemia using blood cultures may be costly, unsustainable, or an inappropriate use of available resources. Development of simple clinical algorithms for identifying high-risk patients who would benefit from sputum smears for AFB, chest radiographs, or from empirical antituberculous therapy might be a better use of resources. For example, HIV-1-infected patients who present with oral thrush, chronic fever, cough or weight loss have a high risk of mycobacteraemia and would benefit from empirical antituberculous therapy and sputum or blood cultures for *M. tuberculosis*.

COMPARISON OF BLOOD CULTURE METHODS

Although the methodologies used for detecting organisms at each of the three study sites were sensitive for recovery of these bacteria, mycobacteria, and fungi, total volumes of blood required for comprehensive cultures were high (20-30 ml), and processing of the Isolator tubes followed by inoculation of the concentrate into Middlebrook broth and onto solid media required additional laboratory steps that are not otherwise necessary during inoculation and processing of blood culture bottles. These additional steps required multiple kinds of culture media and additional laboratory time. Moreover, because of subculture steps, the risk of contamination with environmental organisms is increased using the Isolator tube and lysis-centrifugation system [Campos & Spainhour, 1985; Carey, 1984; Eisenach et al., 1992]. In less-developed countries with limited resources, such added costs are not sustainable. In addition, where clinical microbiology facilities exist for processing mycobacteria blood cultures, testing every patient admitted to hospital may be costly, or require inappropriate use of already scarce microbiology resources. Other factors that preclude the processing of mycobacteria blood cultures in these laboratories include the need for a dedicated carbon dioxide incubator and limited refrigerated facilities for the storage of several kinds of media.

Previous comparative studies have demonstrated that the SC-B system is able to detect more bacterial microorganisms associated with sepsis than conventional broth cultures [Weinstein et al., 1985], and a similar number of bacterial pathogens compared with the BACTEC radiometric system [Weinstein et al., 1986]. Lysis-centrifugation using the Isolator tube has been shown to detect microbiologically proven bacteraemias or fungaemias in significantly greater numbers than either conventional tryptic soy broth bottles with CO_2, or a biphasic bacterial blood culture bottle [Henry et al., 1983]. Direct inoculation of Middlebrook

7H11 conventional solid media or broth media with Isolator concentrate remain two of the most widely used methods for recovery of mycobacteria from blood [Askgaard et al., 1991; Reimer, 1994]. During the conduct of the Tanzania, Thailand, and Malawi studies, inoculating the Isolator concentrate into the SC-AFB biphasic bottle containing Middlebrook 7H9 broth was noted to be more sensitive for recovery of mycobacteria than inoculating the Isolator pellet onto solid Middlebrook 7H11 agar.

For any of the methods presented, culturing blood comprehensively for bacteria, mycobacteria, and fungi requires drawing at least 20-30 ml of blood from adult patients. For cultural reasons, patients in various countries are loathe to part with seemingly large volumes of blood. The data from the Malawi studies indicate that the MFL bottle inoculated with just 5 ml blood is (i) superior to the SC-B bottle inoculated with 10 ml blood, and similar to lysis-centrifugation for detection of bacteraemia and fungaemia; and (ii) superior or similar to Isolator concentrate inoculated on solid media or in Middlebrook broth, respectively, for detection of mycobacteria.

The Malawi study was conducted in a region where nearly three-quarters of febrile adults presenting to the regional general hospital are HIV-1-infected (see Malawi results). During processing, the Isolator/lysis-centrifugation system requires several manipulations that potentially could result in needle stick injury, cuts from broken Isolator tubes, or aerosol exposure to Isolator tube contents. Thus, for laboratory personnel in HIV-1-endemic regions, using the Isolator system increases risk of exposure to the HIV-1 virus. For less-developed world settings, where blood cultures either are or should be part of the microbiology service repertoire, the MFL blood culture system has several advantages: one bottle will yield growth of bacteria, mycobacteria, or fungi, it does not require a carbon dioxide incubator, it is

compact and easily stored, and it is easily read by manually shining an ultraviolet light on the bottom of the bottle. These data demonstrate that comprehensive culture for the three groups of pathogens could be achieved by inoculating just 5 ml of blood into one blood culture bottle rather than inoculating aliquots of blood into various bottles, tubes, or culture media. Ease of a single inoculation into one blood culture bottle circumvents the necessity of multiple inoculations, processing steps, and sub-culturing onto various culture media, thereby reducing rates of contamination, the amount of technician time required for processing, and the risk of needle stick injuries.

The rate of blood culture contamination in Malawi was low (1.6% overall and 0.8% when contamination of Middlebrook 7H11 agar slants were excluded). These low rates were achieved by ensuring that high aseptic standards were maintained before and during the blood draw, using both 70% alcohol and iodine tincture for skin cleansing and allowing the skin to dry properly before venesection, and scrupulous cleaning of the rubber diaphragms of blood culture bottles and Isolator tubes with isopropyl alcohol before inoculation with blood, and before accessing with needle and syringe during microbiologic processing of blood culture bottles and Isolator tubes. Of note, the overall blood culture contamination rate (1.3%) achieved when using alcohol followed by povidone-iodine was similar to the contamination rate (1.6%) observed when skin disinfection was carrried out with alcohol and iodine tincture.

In the United States, the MFL bottle with the BACTEC 9000 series automated machines is marketed as a system for the growth and detection of mycobacteria. Because the MFL blood culture media is not selective and will support the growth of aerobic organisms, such as mycobacteria, bacteria, yeasts, and fungi, the presence of other organisms in the blood may inhibit growth of mycobacteria in the MFL medium. While this might constitute a limitation

in the United States, it is this property that renders the MFL medium, in combination with a hand-held ultraviolet lamp, an excellent system for the comprehensive detection of mycobacteraemia, bacteraemia, or fungaemia in less-developed countries.

In conclusion, one MFL blood culture bottle, inoculated with just 5 ml of blood, was as efficient as the SC-B, SC-AFB, or Isolator blood culture systems in detecting bacteraemia, mycobacteraemia, and fungaemia in a febrile adult population in an HIV-1-endemic region. Moreover, growth in an MFL bottle was easily detected by shining an ultraviolet lamp on the indicator located in the base of the bottle. Such lamps are readily obtained at relatively cheap prices at general electronic stores. The MFL system provides a sensitive and uncomplicated method for comprehensive detection of bloodstream infection in less-developed countries.

UTILITY OF NUMBER OF MFL BLOOD CULTURE BOTTLES IN PATHOGEN RECOVERY

The data in the previous chapter demonstrate that the MFL bottle compares with the gold standard set by the lysis-centrifugation method for detecting bacteraemia, mycobacteraemia, and fungaemia [Henry et al., 1983]. In the present analysis, the data suggest that since nearly all (91%) pairs of bottles had positive cultures following sequential inoculation with blood from a single venipuncture, the culture of a second MFL bottle does not add incrementally to the recovery rate established by inoculation of the first bottle. In fact, the bloodstream infections in 11 (92%) of the 12 patients with mycobacteraemia and 38 (92%) of the 41 patients with bacteraemia would have been detected anyway had only one MFL bottle been inoculated (Table 18). The overall median time to detect bacteria and fungi in the MFL bottles was 1 day (range: 1-4 days). A comparison of the time of recovery for mycobacteria could not be realistically conducted for the two MFL bottles because it was not feasible to determine growth during shipment of the bottles to the United States.

Published recommendations for culture of blood drawn from febrile patients include retrieval of two blood samples, each a minimum of 10 ml, drawn separately and as close as possible to the febrile episode [Reller et al., 1982; Washington, 1986]. These are based on studies conducted in the United States where rates of bloodstream infections are relatively low compared with HIV-endemic settings where bloodstream infection rates among febrile inpatients range from 28% to 48% [see previous thesis chapters]. In the Malawi study, blood from a single venesection was inoculated consecutively into two MFL bottles; the main issue examined in the present analysis was the adequacy of blood volume in a single blood draw for detecting bloodstream infections. The design of the study did not enable ascertainment of the the utility of inoculating blood from two separate blood draws (i.e., two sets) into respective

MFL bottles for bacterial, mycobacterial, or fungal cultures.

Although the practice of obtaining paired bacterial blood cultures from single and separate blood draws has been extended to obtaining mycobacterial blood cultures, there are no published data that critically evaluate these practices. The prevalence rate of bloodstream infections in the Malawi study setting was 25% (95% confidence interval: 19%, 31%).

As outlined in the previous chapter, the MFL bottle is designed for use in the BACTEC 9000 series automated blood culture machines and is licensed and marketed as a system for the growth and detection of mycobacteria [Waite & Woods, 1998]. The MFL blood culture media is not selective and will support the growth of aerobic organisms, such as mycobacteria, bacteria, and yeasts. Microorganisms (e.g., mycobacteria and certain fungi) that grow relatively slowly are less likely to be detected during the first week of incubation. However, since the MFL bottles were kept incubated for a total of 8 weeks, presence of any such pathogens should have been detected. Thus, it is plausible that the predominance of one type of microorganism might have interfered with the recovery of other types and therefore prevent detection of polymicrobial (i.e., >1 pathogen) bloodstream infections. This may explain why none of the MFL pairs exhibited any evidence of polymicrobial bloodstream infections.

In summary, aseptic inoculation of 5 ml of blood into one MFL bottle will detect about 92% of cases of bacteraemia and mycobacteraemia. In addition, the data suggest that the MFL bottle serves as a useful, multipurpose tool for the detection and characterization of bloodstream infections in settings of HIV endemicity in less-developed countries and that inoculation of blood obtained in a single blood draw into a single MFL bottle is acceptable for detecting bacteraemia and mycobacteraemia. A second MFL bottle does not significantly increase the sensitivity of detection of these pathogens.

EMERGING PATHOGENS: SAV GROUP MYCOBACTERIA

Advances in laboratory methodology have enabled more rapid and reliable differentiation of mycobacterial species more commonly associated with clinical illness (e.g., *M. tuberculosis* and *Mycobacterium avium* complex), and the identification of new or emerging species (e.g., *M. triplex*) [Hale et al., 2001]. However, ambiguities in determining specific mycobacteria species remain, and methods for identification and susceptibility testing are not standardized for many species. The mycobacterial isolates obtained from the four patients with SAV Group mycobacteraemia were tested using similar methods by two different References Laboratories with extensive experience in working with mycobacteria. Standard susceptibility testing panels have not yet been established for these microorganisms.

These features may lead to difficulties in the clinical management of patients with disseminated mycobacterial infection. The clinical manifestations of such infection are relatively nonspecific and not indicative of the infecting species. The four patients with SAV Group mycobacteraemia had presented with typical, nonspecific clinical features, such as fever, lymphadenopathy, and cachexia that also are commonly seen in patients with disseminated mycobacterial infections caused by *Mycobacterium avium* complex or *M. tuberculosis*. There were no distinctive clinical findings among these patients that would aid in making a specific diagnosis of *M. simiae* or SAV mycobacterial infection. Thus, as with other mycobacterial infections, diagnosis and specific therapy is ideally guided by laboratory testing, including species identification and susceptibility testing, rather than clinical findings alone.

Because the primary focus of these studies was bloodstream infections in general rather than bloodstream infections in HIV-1-infected patients specifically, neither CD4 lymphocyte

nor HIV-1 viral load data were obtained from patients in these studies. However, since each of the four patients had a marker of symptomatic HIV-1 infection (oral candidiasis, Kaposi's sarcoma, or positive cerebrospinal fluid cryptococcal antigen), it can be assumed that all had at least moderately advanced disease. In addition, because these patients had at least moderately advanced HIV-1 infection and clinical evidence of probable other opportunistic infection, and because the symptoms and physical findings were nonspecific, one could not be certain that *M. simiae* or SAV mycobacteria were the cause of their symptoms. Further study and characterization of SAV mycobacteria and of the clinical illness with which it is associated are required to better ascertain the frequency and clinical significance of these mycobacterial infections. No data on treatment or post-discharge outcome for these patients were available.

Awareness of *M. simiae* and SAV mycobacteria as potential causes of disseminated infection in patients with AIDS is of public health importance for several reasons. Because of their phenotypic similarity to each other and to other mycobacterial species, patients infected with *M. simiae* or SAV mycobacteria may go unrecognised and presumed to be infected with other mycobacterial species, particularly *M. tuberculosis,* in resource-poor settings without access to adequate laboratory testing. This may lead to ineffective treatment, as not all species are susceptible to all agents (testing of three isolates using the methods established for *M. tuberculosis* suggests resistance to many antituberculosis drugs). Lastly, had it been assumed that these SAV mycobacteria were *M. tuberculosis*, the presence of MDRTB would have been overestimated. Because of the lack of data and clinical experience with *M. simiae* and SAV group infections, the best definitive treatment has yet to be determined. Infections with other mycobacteria, particularly *M. tuberculosis*, require treatment with multiple agents to which the organisms are susceptible for prolonged periods. Not adhering to these principles may

promote the development of drug-resistance. It is unknown at present whether similar hazards exist when SAV mycobacteria are treated with ineffective agents or otherwise suboptimal therapy.

CHAPTER 9

LIMITATIONS

Only one set of blood culture (i.e., blood from one venipuncture) was drawn from each study patient; two blood cultures would probably have yielded a larger number of organisms [Weinstein et al., 1983]. It follows that the true rates of bloodstream infections in the study populations were probably higher than the observed rate of 28% in Tanzania, 48% in Thailand, and 28% in Malawi.

Blood cultures could not be repeated in some patients in whom *S. epidermidis*, *Micrococcus* spp, and *Bacillus* species were isolated because the patients died before the organisms were identified in the laboratory. The decision to treat these organisms as contaminants was based on the results of studies work completed in the United States that suggest the presence of these organisms in the blood does not correlate with presentation and clinical state [Weinstein et al., 1983].

The predominance of one organism in the blood may interfere with the recovery of other types and therefore prevent the detection of polymicrobial bloodstream infection.

HIV-1 ELISA results were not confirmed by secondary tests. Data from Tanzania and Thailand have shown that the use of two ELISA tests to confirm the presence of HIV-1 antibodies produces results comparable to those of the Western blot [Urassa et al., 1992; Ittiravivongs et al., 1996]. Thus, in a country with a high prevalence rate of HIV-1 infection and limited financial resources, Western blot analysis is neither customary nor necessary, though second less sensitive, more specific immunoassays are recommended for confirmation in Western countries.

Different body sites and fluids were not cultured for pathogens. It was therefore not

possible to correlate the presence of a bloodstream infection with a concurrent positive culture for the same organism from another anatomic site.

Although the HIV test kits used in these series of studies were able to detect HIV-2 antibody, the design of the study did not enable ascertainment of specific patients with HIV-2 antibody. To date, however, no HIV-2 infection has been found in Tanzania or Malawi despite continuous search by researchers.

The patient populations under study had remarkably high HIV-1 seroprevalence rates. This underscores the potential pitfalls in applying these results to other populations that may have a relatively lower HIV-1 seroprevalence rate, and the need for region-specific studies. The results of a two-month survey from one hospital are not representative of other regions for any of the countries where the studies were conducted because the aetiology of bloodstream infections and the clinical predictors of bloodstream infection using logistic regression were highly variable. This may be due to seasons, geography, the environment, available diagnostic microbiology resources, and the susceptibility of the patient population. For clinical predictors to be useful, they would have to be assessed for the area in which they would potentially be used. One cannot extrapolate the results of a four week study in Lilongwe, Malawi to other regions in sub-Saharan Africa, or to other countries. Thus, the predictors that were ascertained through multivariate analysis are useful primarily as an example. Appropriate clinical algorithms would have to be developed from studies conducted in the respective regions. For clinical predictors to be useful, they would have to be determined for patient populations in regions where they would be used.

The Malawi studies were conducted during the height of the Malawi dry and wet seasons and confirmed seasonal variation in the occurrence of salmonella and pneumococcal

bloodstream infections and malaria. In contrast, the studies in Tanzania and Thailand were conducted during only one cycle of seasons. Thus, the data from Tanzania and Thailand do not enable similar ascertainment of the role of seasons on the nature and occurrence of bloodstream infections. Confirmatory assessments of seasonal variations in the aetiology of bloodstream infection in hospitalized populations may need to be determined over several seasonal cycles in some countries.

High rates of malnutrition and HIV-1 infection (both of which may suppress the febrile response) were documented for the study populations in Tanzania, Thailand and Malawi. Thus, using fever as the case definition for patient enrollment might have excluded a significant number of afebrile, HIV-1-infected patients who had bloodstream infections.

The type of antimicrobials used by some study-patients before presentation to hospital and the duration of such therapy were often uncertain. Moreover, the study populations had relatively high rates of pre-admission antimicrobial exposure. In Malawi, antimicrobial exposure during the wet season was lower than in the dry season rate (35% wet season vs. 48% dry season; $P < 0.01$). However, this significant seasonal difference in antibacterial exposure preceding hospital admission did not appear to significantly affect the percentage recovery of bloodstream infection pathogens (29% wet season *vs* 28% dry season; P = NS). Although the data suggest that exposure to antimicrobials before hospital admission was not associated with the presence or absence of bloodstream infection, there was documentation of resistance among the non-*typhi Salmonellae* isolates to two antimicrobial agents that are commonly prescribed at the Malawi study site. Thus, these data further emphasize the need to control unrestricted antimicrobial use in international settings if emerging antimicrobial resistance is to be prevented.

There were limitations to the analysis of the blood culture methods. For example, while the MFL blood culture system was as sensitive as the Isolator concentrate inoculated on IMA in detecting fungi, the numbers of these isolates (11 *C. neoformans*, one *Cryptococcus laurentii*, and one *Candida tropicalis*) were too small to enable a valid assessment of overall sensitivity of fungi. For similar reasons, Waite et al also were not able to make any general inferences about the reliability of the MFL bottle for the recovery of fungi [Waite et al., 1998]. Moreover, the numbers of fungal bloodstream infections were too small to enable a valid assessment of one versus two MFL bottles in detecting fungaemia

It was demonstrated that the MFL bottle's ability to recover bacteria, mycobacteria, and fungi was comparable to the lysis-centrifugation method. However, comparison of the MFL bottle with other bottles, such as the BACTEC™ bottles containing large volumes of resin, has not been conducted in similar settings with high rates of HIV-1 and bacteraemia.

Because the MFL and SC-AFB bottles and the Middlebrook 7H11 agar slants were shipped to the United States for further processing, assessment and comparison of relative times of detection of mycobacteria by these respective recovery methods were not possible. As expected for most comparative studies of this type, there was a bias as regards the volume of blood inoculated into the various media. Ten ml of blood were inoculated into each of the SC-B bottle and Isolator tube; 5 ml were inoculated into the MFL bottle as recommended by the manufacturer. Thus, it is likely that the recovery rate of pathogens from the MFL system might have been higher had the inoculating volume of blood into MFL bottles been >5 ml.

CHAPTER 10

CONCLUSION

INSIGHTS INTO EFFECTIVE DEVELOPMENT AND USE OF MICROBIOLOGY LABORATORY DIAGNOSTICS IN DEVELOPING COUNTRIES

Using the experience of the approach that was used to study bloodstream infections in Tanzania, Thailand, and Malawi, the issue of limited microbiology resources in developing countries was reviewed. This conclusion includes a discussion of the feasibility and utility of the cohort-based approach to integrate microbiology, epidemiology, and clinical medicine to survey emerging bloodstream infections in these countries, and the role of sentinel hospitals in carrying out these endeavours.

In industrialized countries, it is the best of times for microbiologic diagnosis and treatment of infections. In some developing countries, progress is also apparent. Ministries of health are building hospital intensive care units (ICUs) with increasing use of sophisticated medical devices, and invasive diagnostic and therapeutic procedures and interventions. Increasing numbers of infants and adults are being admitted to, and benefiting from, these units. More patients with conditions such as chronic renal failure or hematologic disorders are being treated in specialized units. The Internet has made physicians generally more knowledgeable than before.

Nevertheless, it is the worst of times for hospitals in other developing countries, where infectious diseases remain the leading cause of death [Hinman, 1998; Mabey et al., 2004]. Many sentinel hospitals have less than basic microbiology laboratory facilities; there is no end in sight to the HIV epidemic, and the rate of tuberculosis is increasing in parallel with it;

hospital infections, especially surgical site infections, have become important causes of illness and death in certain hospitals in sub-Saharan Africa (unpublished data); and invasive medical devices and procedures are increasingly being introduced into ICUs and operating theatres without the necessary infection control procedures. In some developing countries, some institutions have all the needed microbiologic resources, while others have none; some hospital laboratories have instruments and reagents yet have no technical staff to use them; others may be able to amplify genomes yet cannot report the results of a simple Gram stain in a timely manner. For all these reasons, the causes of many infections among inpatients in Africa, Southeast Asia, the Indian subcontinent, and parts of the Americas remain largely unknown or uncharacterised.

In sub-Saharan Africa and Southeast Asia, antimicrobial-drug resistance is being increasingly recognised in pathogens that commonly cause infections in health-care settings, rendering available antimicrobial agents ineffective and further shortening the list of already scarce effective agents [Hart & Kariuki, 1998]. Thus, to diagnose and treat infections appropriately and to fully characterize emerging infections in developing countries, enhanced clinical microbiology services should be a priority. The clinical microbiology laboratory in developing countries should be patient directed and guided by clinical reality and not by high technology or outside interests.

Two other factors have had a marked effect on the role of clinical microbiology in developing countries, the HIV and tuberculosis epidemics. Most (95% of the global total) people with HIV infection live in the developing world [Grant & De Cock, 1998; World Health Organization, 1999]. In almost 6 million of the 34 million adults and children with HIV or AIDS, HIV infection was acquired during 1999 [World Health Organization, 1999];

3.8 million cases occurred in sub-Saharan Africa and 1.3 million in South and Southeast Asia. Of the approximately 40 million Tuberculosis cases globally, 73% are projected to have occurred in Southeast Asia and sub-Saharan Africa [World Health Organization, 2000]. Tuberculosis, which accounts for almost one third of the AIDS deaths worldwide, and other opportunistic bacterial, fungal, and protozoal infections are leading causes of death among HIV-infected patients [Grant & De Cock, 1998; World Health Organization, 1999; World Health Organization, 2000]. In consequence, HIV infection, tuberculosis, and HIV-related opportunistic infections have overwhelmed existing resources in hospital microbiology laboratories in most developing nations.

At CDC, a main objective of the strategy for preventing and controlling emerging infectious diseases in developing countries is establishing more effective international surveillance networks [Centres for Disease Control and Prevention, 1994]. In the industrialized world, infection control relies on results from individual patient-directed diagnostic microbiology laboratory tests. However, basic clinical microbiology has not been recognised as a priority by donor or governmental agencies in industrialized countries or by the developing countries themselves. The problem often has been compounded by lack of trained laboratory personnel or prohibitive costs associated with maintaining a laboratory. Where resources are available, they may be used inappropriately (e.g., nonessential stool, urine, or sputum cultures; antimicrobial susceptibility testing of microorganisms without quality assurance; or complete laboratory characterization and antimicrobial susceptibility testing of bacterial isolates that are not clinically relevant).

Prohibitive costs and doubtful cost-effectiveness of specific tests are commonly cited as reasons for the unavailability of microbiology tests. The first steps in achieving cost-effective

use of resources include assessing whether or not a test has sufficient diagnostic value to be used and establishing criteria to limit processing to those organisms most likely to be clinically relevant [Robinson, 1994]. The concept of clinical value encompasses several issues [Spencely et al., 1979]: why was the test requested? Will the result help or alter patient management? Would a simpler test do? Will the use of a test increase knowledge? Can we do without it? Is the test of public health or clinical importance? For example, hospitals in developing countries still routinely obtain and process anaerobic blood cultures, despite that a positive anaerobic blood culture often reflects an underlying anaerobic infection (e.g., intraabdominal sepsis or female genital tract infection) that already is clinically apparent or discernible [Ortiz & Sande, 2000; Bartlett & Dick, 2000]. The counter argument is that while such data may reflect reality for a microbiology issue in industrialized nations, they may not be applicable for developing settings—all the more reason for important questions about diagnostic clinical microbiology in developing countries to be addressed through evidence-based clinical studies.

The importance of integrating epidemiology and microbiology is exemplified by studies that ascertained the usefulness of expensive HIV confirmatory tests in developing countries. In industrialized countries, confirmation of HIV serologic tests with the Western blot molecular technique is standard practice. In developing countries, the Western blot often is not routinely used because of its complexity and high cost. Studies with epidemiologic, clinical, and microbiologic components that were conducted in Thailand and Tanzania have shown that the use of two ELISA assays to confirm the presence of HIV antibodies produces results comparable with those of the Western blot [Urassa et al., 1992; Ittiravivongs et al., 1996]. This approach was effectively used to confirm HIV status of patients in the present

study protocol in HIV-endemic regions in Tanzania, Thailand, and Malawi. Thus, in a country with a high prevalence rate of HIV infection, limited financial resources, and inadequate laboratory infrastructure, Western blot analysis for confirmation of HIV infection is not essential.

Medical services in industrialized nations rely on results from individual, patient-directed, diagnostic microbiology laboratory tests ordered by clinicians. This system appears effective for industrialized settings and is generally sustainable. Not surprisingly, diagnostic microbiology services in some developing countries have been modelled on these practices in industrialized countries. However, such routine laboratory testing may be impossible in developing settings because of lack of microbiology services, or, where these services are available, tests may be unreliable if performed improperly or without adequate quality control. Further, the tests may well be inappropriate, irrelevant, or redundant. For example, antimicrobial susceptibility testing without quality controls may lead to invalid or distorted data that give rise to bias and inaccuracy in reports being used for clinical and public health decision making.

HOSPITAL COHORT-BASED STUDIES

The study protocol for the present thesis is, in fact, a series of hospital, cohort-based, epidemiologic and microbiologic surveys. Such surveys are conducted with a cohort of patients who meet simple, objective entry criteria or case definitions (e.g., fever, diarrhoea, cellulitis, or specific syndromes). Detailed clinical and epidemiologic data are collected for later analyses, and cultures with a high positive predictive value for infection (e.g., blood, cerebrospinal fluid, other sterile sites, or stool for enteric pathogens) are obtained. The

emphasis is on performing quality-controlled laboratory testing for a finite period rather than long-term, routine diagnostic testing. These surveys are best conducted in selected hospitals or laboratories that provide a natural gathering point to sample patients meeting these entry criteria. A cohort-based study carried out during a finite time period may be more effective than long-term individual patient-directed laboratory testing in providing useful clinical and public health information, in determining the true incidence and prevalence rates of emerging pathogens and antimicrobial-drug resistance, and in yielding clinical predictors for various infections in defined patient cohorts. In addition, cohort-based studies provide the opportunity to establish diagnostic capability in basic clinical microbiology in sentinel hospitals or laboratories and promote surveillance activities in regions where critical public health infrastructures have been neglected.

COHORT STUDIES OF BLOODSTREAM INFECTION

Using the cohort-based approach to clinical microbiology and epidemiology, studies of bloodstream infections among inpatients in sub-Saharan Africa and Southeast Asia were executed and completed. Fever was chosen as the case definition because it may be attributed to HIV infection, diarrhoea, pneumonia, tuberculosis, or, in sub-Saharan Africa, malaria. Blood cultures were obtained because of their high positive predictive value for presence of bloodstream infections in febrile patients.

In Thailand, about half of consecutive febrile adults admitted to a sentinel teaching hospital for infectious diseases had bloodstream infections; in similar patient cohorts in Tanzania and Malawi teaching hospitals, over one quarter of patients had a bloodstream infection. For these three countries, *M. tuberculosis, S. pneumoniae*, and *Salmonella* species

were the predominant causes of bloodstream infections in these patients. Data from these studies also included clinical predictors for bloodstream infections and antimicrobial susceptibility profiles of clinically important isolates, including *M. tuberculosis* isolates. Both the predictors and susceptibility profiles were potentially useful for developing algorithms for empiric treatment of febrile inpatients and for helping clinicians decide which patients would most benefit from limited blood culture services, where these were available.

The cohort-based studies in Malawi during the dry and wet seasons demonstrated seasonal variation in the prevalence of bloodstream infections: *S. pneumoniae* and *M. tuberculosis* were the predominant bloodstream pathogens during the dry season, whereas *Salmonella* species were the predominant bacteria isolated during the wet season. In addition, the Malawi data confirmed that malaria was overdiagnosed in both the wet and dry seasons in Malawi and that empirical therapeutic decisions had to reflect this reality.

The cohort-based studies in Tanzania, Thailand, and Malawi demonstrated the occurrence of occult mycobacteraemia and new emerging pathogens. For example, 42% of patients with *M. tuberculosis* bloodstream infections had neither symptoms nor signs of pulmonary tuberculosis. These results highlighted the importance of maintaining a low threshold of suspicion for active tuberculosis; the need for strengthening each hospital's microbiology capabilities to examine and report on sputum smears for AFB; and the potential for intrahospital tuberculosis transmission from seemingly noninfectious patients.

The public health implications of the cohort-based approach are enormous. Conducting similar studies in other countries would improve microbiology services by encouraging appropriate use of limited resources in sentinel hospital laboratories and focussing on clinically relevant problems (e.g., bloodstream infections, meningitis, pneumonia, febrile

diarrhoea, and surgical wounds). Moreover, laboratory personnel would benefit from training in the conduct of quality-controlled tests, such as antimicrobial-drug susceptibility testing. Prevalence rates of common infections, HIV infection, or resistance of common hospital pathogens to available antimicrobial agents would be available for clinical and public health decision making. Updated lists of probable diagnoses, clinical predictors for specific infections, and development of clinical algorithms and antimicrobial-drug susceptibility profiles based on these objective data would enhance patient care through rational diagnosis and prescribing policies.

Although it may not be economically feasible to obtain cultures for all patients who might benefit from microbiologic tests in developing countries, cohort-based studies could be applied to establish the causes and clinical predictors for these infections and thereby facilitate directed rather than blind empirical therapy. Data from cohort-based studies in one region or country may not be suitable for direct extrapolation to other regions or countries. Rather, regional, season-specific surveillance studies can be tools for optimizing patient care where routine laboratory testing is not available. The data from Thailand and Malawi also included documentation of bloodstream infections caused by a new emerging pathogen: SAV Group mycobacteria. The task remains to define the role of other new and emerging pathogens in various patient populations at hospitals in developing countries, using the cohort-based approach.

THE ROLE OF SENTINEL HOSPITALS

The cohort-based approach to collaborative global endeavours in the characterization of emerging infections has included identifying sentinel hospitals and then enhancing their

clinical microbiology laboratory capacity by assessments, training of personnel, and initiating projects that answer questions of clinical and public health significance. In developing countries, where limited resources and infrastructure may preclude comprehensive medical, surgical, and laboratory services for every region or province, centralization of available resources in a few selected centres is one way of optimizing resources. This paradigm is evident in many countries in Southeast Asia, Africa, Latin America, and the Caribbean, where a few institutions have evolved into sentinel centres of paramount importance for providing such services.

Sentinel hospitals tend to be large institutions (usually >500 beds) that are the main teaching centres for medicine, surgery, nursing, and laboratory science; they commonly house specialized ICES, surgery, haemodialysis, or invasive medical procedures; they have problems with hospital infections and antimicrobial-drug resistance; they are associated with microbiology laboratories that are often reference centres with the ability and capacity to conduct various microbiologic tests using scrupulous, quality-controlled methods; and they usually are government affiliated and have very close links with the respective ministry of health. The last attribute is important since governmental agencies from industrialized countries (e.g., WHO, United States Agency for International Development) generally prefer to maintain collaborative endeavours with sentinel centres for reasons that include adequate infrastructure, trained personnel, and access to the ministry of health.

A high priority for future global consortiums of epidemiology and biomedical research centres will be to initiate or build upon existing systems in sentinel hospitals in developing countries for the international monitoring and reporting of antimicrobial susceptibility data. Two systems that offer a foundation of international linkages are CDC's International

Nosocomial Surveillance Program for Emerging Antimicrobial Resistance and the WHO Antimicrobial Resistance Monitoring Program. The international and national objectives of these programs depend on conducting proper, quality-controlled, antimicrobial susceptibility testing and promoting the use of resistance data to guide antimicrobial therapy. These results, when integrated with clinical and epidemiologic data on opportunistic and hospital infections, may lead to substantial improvement in patient outcomes.

APPENDIX A

MAP OF TANZANIA

APPENDIX B

MAP OF THAILAND

APPENDIX C

MAP OF MALAWI

APPENDIX D

			Data Entry
Study Number [ ][ ][ ] Ward_____			NO [ ] WD [ ]
Admission Date___/___/___ Hospital number_____			
Gender [1] Male [2] Female Age_____			GEN [ ] AGE [ ]
Occupation_____ Family size_____			OCC [ ] FAM [ ]
No. rooms in house_____			ROOMS [ ]
***1.* History**			
Presentation_____			ADMDX [ ]
Date of onset _____/_____/_____			ONSET [ ]
Temperature _____°C _____°F			BTC [ ] BTF [ ]
Respiratory rate____/min			RR [ ]
Pulse_____/min BP: _____/_____			P [ ] BP [ ]
***2.* Acute Symptoms**			SFEV [ ]
1) Fever	[1] Yes	[2] No	SCHI [ ]
2) Cold/clammy (chills)	[1] Yes	[2] No	SCOU [ ]
3) Cough	[1] Yes	[2] No	SSOB [ ]
4) Shortness of breath	[1] Yes	[2] No	SDIA [ ]
5) Diarrhoea	[1] Yes	[2] No	SVOM [ ]
6) Vomiting	[1] Yes	[2] No	SCONV [ ]
7) Convulsions/LOC	[1] Yes	[2] No	HEADACHE [ ]
8) Headache	[1] Yes	[2] No	NECKSTIF [ ]
9) Neck stiffness	[1] Yes	[2] No	SKINLES [ ]
10) Infected Skin Lesion(s)	[1] Yes	[2] No	JAUND [ ]
11) Jaundice	[1] Yes	[2] No	GENULCER [ ]
12) Genital ulcers	[1] Yes	[2] No	URIN [ ]
13) Urinary symptoms	[1] Yes	[2] No	RASH [ ]
14) Rash	[1] Yes	[2] No	FAMCOUGH [ ]
Family history of cough	[1] Yes	[2] No	PMH [ ]
Past medical history (PMH).....		[ ]	
***3.* Chronic Symptoms (over one month)**	[1] Yes	[2] No	CSYM [ ]
1) Fever	[1] Yes	[2] No	CFEV [ ]
2) Cough	[1] Yes	[2] No	CCOU [ ]
3) Diarrhoea	[1] Yes	[2] No	CDIA [ ]
4) Weight loss	[1] Yes	[2] No	CAC [ ]
5) Chronic Skin Lesion(s)	[1] Yes	[2] No	CSL [ ]
6) Vomiting	[1] Yes	[2] No	CVOM [ ]

APPENDIX D (continued)

***4.* Physical Examination**	ORAC [ ]
1) Oral candidiasis [1] Yes [2] No	LEUKO [ ]
2) Oral leukoplakia [1] Yes [2] No	KAP [ ]
3) Kaposi sarcoma [1] Oral [2] No [3] Both [4] Skin	ADENOP [ ]
4) Lymphadenopathy [1] Yes [2] No	HEPENL [ ]
5) Hepatomegaly [1] Yes [2] No	SPLEENL [ ]
6) Splenomegaly [1] Yes [2] No	BCGSCA [ ]
7) BCG scar [1] Yes [2] No	ZOSCAR [ ]
8) Zoster scar [1] Yes [2] No	CRACK [ ]
9) Chest: i)Crackles [1] Yes [2] No	EFFUS [ ]
ii) Effusion [1] Yes [2] No	CONSOL [ ]
iii) Consolidation [1] Yes [2] No	SIMPLEX [ ]
10) Herpes simplex [1] Acute [2] No [3] Chronic	NECKRIG [ ]
12) Neck rigidity [1] Yes [2] No	CNS [ ]
10) Central nervous system [1] Confused [2] Alert [3] Unconscious	RASH [ ]
11) Rash [1] Yes [2] No	
***5.* Medications (pre-admission)**	
1) Antibiotics [1] Yes [2] No	ABX [ ]
2) Antifungals [1] Yes [2] No	AFG [ ]
3) Antimalarials [1] Yes [2] No	AMAL [ ]
4) Anti TB drugs [1] Yes [2] No	ATUB [ ]
***6.* Laboratory**	
1) Hb_____	HB [ ]
2) WBC_____	WBC [ ]
3) Malaria smear [1] Positive [2] Negative [3] Not done	MAL [ ]
4) Chest X-ray [1] Abnormal [2] Normal [3] Not done	CXR [ ]
5) Sputum AFB [1] Positive [2] Negative [3] Not done	SAFB [ ]
6) CSF results [1] Abnormal [2] Normal [3] Not done	CSF [ ]
7) HIV status [1] Positive [2] Negative	HIV [ ]
***7.* Discharge Date _____/_____/_____**	DD [ ]
1)DIAGNOSIS_____	DIAG [ ]
2) Outcome [1] Discharged [2] Died [3] Still Inpatient [4] Absconded	OUTC [ ]
3) Length of hospital stay _____ days	LOS [ ]

APPENDIX E

CONSENT FORM FOR ADULT PATIENTS WITH FEVER

You are being asked to be in a research study of bloodstream infections. Bloodstream infections mean that microorganisms, or germs, have gotten into the blood. These germs should not be there and these infections are serious. People often get a fever with the infection. Research studies already done in Africa, Southeast Asia, Europe and the United States have shown that in people admitted to hospital with fever, bloodstream infections are more common than we thought. Also, the germ which causes Tuberculosis has been found to be a common cause of bloodstream infections in people with fever who are infected with the AIDS virus. We are trying to find out how many people in Tanzania, Malawi, and Thailand who come to hospital and have a fever, have a bloodstream infection. We are asking people at Muhimbili Medical Centre (Tanzania), Bamrasnaradura Hospital (Thailand), and Lilongwe Central Hospital (Malawi) to be in this study. The Ministry of Health in these three countries, a public health organization from the United States, the Centres for Disease Control and Prevention (CDC), and Duke University Medical Center in the United States are doing the study.

We are asking you to be in this study because you have been admitted to hospital and you have a fever. If you decide to be in this study, you will be asked a few questions about your illness, and then you will have a physical examination. After this, we will take about 25 ml of blood (5 teaspoonfuls) from you: 10 ml will be cultured for germs, including *Mycobacterium tuberculosis*, the organism which causes tuberculosis; 5 ml will be used for checking your blood count and for malaria; 5 ml will used for testing of HIV-1, the virus that causes AIDS; and 5 ml will be used to test how well your body fights infections by testing your immune

response to germs that might be in the blood. These are all tests routinely performed on patients with a fever in the United States who might have been infected with the AIDS virus. What follows is more detail on each part of this study. There are four parts:

Part #1

Blood cultures. Taking blood by needle puncture so that we can try to grow germs from it (we call this "culture") is a routine medical procedure. Doctors usually give patients with a fever, like you, antibiotics. The choice of antibiotics which you receive depends on which germs are in the blood. Identifying germs in your blood and sharing this information with your doctor will allow the doctors taking care of you to make better choices of antibiotics to treat an infection, if you have one. If blood cultures are not done, a bloodstream infection will not be found and you may not receive the right therapy. These germs will also be sent to Duke University in the United States, to see if they get the same results we do here.

Part #2

HIV tests. Doctors would like to know if patients with fever, who have germs in their blood, are infected with the AIDS virus. Also, this information will help in planning your medical treatment. The blood test that is used in this hospital to tell whether a person is infected with the AIDS virus is called the HIV antibody test. Antibodies are substances made by the blood to fight infection. When a person is infected with the AIDS virus, the body produces antibodies against HIV. If antibodies are present in the blood, which is then tested by the HIV antibody test, the test will be positive. If there are no antibodies in the blood, the test will be negative. If there are antibodies in the blood, tests may be done to measure how much HIV

virus is in the blood. A positive HIV antibody test means that a person is infected with the AIDS virus. It also means that this person can give the AIDS virus to other people. The AIDS virus is found in the blood, semen, and vaginal fluids. A person can get the AIDS virus by having sex with someone who is infected. Women who are pregnant can also give the AIDS virus to their unborn babies. A person cannot get the AIDS virus by holding hands, hugging, drinking from the same glass, or being in the same room with someone who has the AIDS virus. A person recently infected with the AIDS virus may not yet have a positive HIV antibody test since it sometimes takes 3-6 months after infection for antibodies against the AIDS virus to show up in a blood test. However, during this early period, when the test is not yet positive, the recently infected person can still infect others with the AIDS virus. In addition, having a negative AIDS test now does not protect against having infection with the AIDS virus in the future. The HIV test can provide information that can help your doctor treat you and help you stay healthy and avoid giving the HIV virus to others. For this reason, doctors and nurses recommend the test to patients like you whose illnesses suggest the possibility of AIDS.

As recommended by the Ministry of Health, blood will be tested for HIV only after you have received counselling about the test and about what it means to be infected, or not infected. You will also receive counselling once the HIV test results are done, whether the test is positive or negative. Counselling will be carried out by permanent staff counsellors from the adult medical wards.

Part #3

Other blood tests. Your blood will be tested for malaria which also causes of fever.

Part #4

Leftover serum samples. After all the above tests have been done, leftover serum will be stored in freezers in Dar es Salaam, Bangkok, and Lilongwe. Should the results of all your tests prove negative, having the stored serum will enable us to check for other agents (e.g., influenza virus) that could have been responsible for your fever. These results will be under the authority of the doctor who was responsible for your care during your hospitalization. Your name will be removed from all data collection forms. The tube with your blood will have a code number so that your identity will not be revealed. No individual identities of patients will be forwarded to CDC or Duke University Medical Center.

Confidentiality

We will keep the information we get from you as private as we can. Your name will not be on your test results–we will use a number instead of your name. The results of your blood culture, HIV antibody test, and other blood tests will be told only to your doctor. These results will be under the authority and supervision of the doctor responsible for your inpatient care. Your doctor will discuss these results with you. Your doctor might write the results of your HIV test in your medical notes. All this information will be kept confidential in your medical records to the extent legally possible. Your name will not be mentioned in any papers or speeches about the study.

Risks

There are several possible risks to you from being in this study. First, taking blood by needle will hurt for a moment, and may leave a bruise. Second, having an HIV test might cause you

some anxiety and, if your test is positive, this will likely change the way you think about the future. Third, there is the chance that someone outside the hospital may find out that you have been tested for HIV infection or may find out the results of your HIV test, although we think this is a small chance.

Benefits

There are several benefits to you from being in this study. First, if you have a bloodstream infection, your doctor will better be able to treat your infection. Second, if you have a low blood count, your doctors might decide to offer you a transfusion. Third, many people want to have an HIV test, because it helps them plan for the future and, if it is positive, help keep them from infecting others. If your test is positive, your doctors will be better able to manage your illness, also. Last, you will be helping other people in the future, because the results of this study will allow doctors to better treat people who have illnesses like you.

Costs

There will be no cost to you.

Refusal to participate

You may refuse to be in one or all parts of the study. If you do not want to be in the study or to have an HIV antibody test, that decision will not in any way interfere with your ability to receive proper medical care or attention. If you do have a bloodstream infection but choose not to be in the study, it is highly likely that this bloodstream infection will not be detected. Because you are ill, your doctor might still treat you with antibiotics but may not select the

correct one to treat the germ that is making you ill. Should you choose to not be in the study but your doctor asks that the research investigators get blood cultures from you on the grounds that the information will help him/her decide the course of your medical management and therapy, we would be very happy to do so with your consent.

Contact information

1) If you have questions about the study, please contact:

.....

Telephone number:.....

(2) If you have questions about your rights as a participant of this study please contact:

Name: .....

Telephone number:.....

(3) If you sustain any complications as a result of venipuncture please contact:

.....

Telephone number: .....

Summary statement

I have been told that my being in the study is up to me, that I choose to be in only part of it, and that I can stop being in the study at any time without any change in the medical care I would get if I were not a participant in the study. The doctor has told me about blood cultures, HIV antibody testing, and has explained the other blood tests. I have been able to ask questions about blood cultures, the clinical importance of bloodstream infections, HIV testing, and other immunologic testing.

Do you:

Agree to blood cultures (Part 1): Yes..... No.....

Agree to HIV testing (Part 2): Yes..... No.....

Agree to other blood tests (Parts 3 and 4) Yes..... No.....

Name of patient .....

Relative or person to contact if there is a complication: .....

Signature of patient.....

Name of physician advising patient.....

Signature of physician .....

Witness for oral consent.....

By signing below, I attest that the consent form was accurately read to the participant

Signature of witness for oral consent.....

Date .....

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Fatal *Mycobacterium tuberculosis* Bloodstream Infections in Febrile Hospitalized Adults in Dar es Salaam, Tanzania

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Fever and Human Immunodeficiency Virus Infection as Sentinels for Emerging Mycobacterial and Fungal Bloodstream Infections in Hospitalized Patients ≥15 Years Old, Bangkok

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A Hospital-Based Prevalence Survey of Bloodstream Infections in Febrile Patients in Malawi: Implications for Diagnosis and Therapy

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Comparison of BACTEC MYCO/F LYTIC and WAMPOLE ISOLATOR 10 (Lysis-Centrifugation) Systems for Detection of Bacteremia, Mycobacteremia, and Fungemia in a Developing Country

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Utility of Paired BACTEC MYCO/F LYTIC Blood Culture Vials for Detection of Bacteremia, Mycobacteremia, and Fungemia

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