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Surveillance programs for detection and characterization of emergent pathogens and antimicrobial resistance. Results from the Division of Infectious Diseases, UNIFESP

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ABSTRACT

Several epidemiological changes have occurred in the pattern of nosocomial and community acquired infectious diseases during the past 25 years. Social and demographic changes possibly related to this phenomenon include a rapid population growth, the increase in urban migration and movement across international borders by tourists and immigrants, alterations in the habitats of animals and arthropods that transmit disease, as well as the raise of patients with impaired host defense abilities. Continuous surveillance programs of emergent pathogens and antimicrobial resistance are warranted for detecting in real time new pathogens, as well as to characterize molecular mechanisms of resistance. In order to become more effective, surveillance programs of emergent pathogens should be organized as a multicenter laboratory network connected to the main public and private infection control centers. Microbiological data should be integrated to guide therapy, adapting therapy to local ecology and resistance patterns. This paper presents an overview of data generated by the Division of Infectious Diseases, Federal University of São Paulo, along with its participation in different surveillance programs of nosocomial and community acquired infectious diseases.

Key words: emerging infectious diseases, HIV, AIDS, candidemia, antimicrobial resistance, bacteremia, sepsis, nosocomial infectious.

INTRODUCTION

Since the development of antimicrobial drugs against bacteria, fungi, virus and protozoa, resistance has been a major concern, particularly after the widespread use of antibiotics in clinical settings. Failure of conventional therapy of infectious diseases is obviously responsible for high morbidity and mortality rates in different populations, in addition to the increase of costs of health assistance, particularly when new and generally highly expensive antimicrobial drugs need to be incorporated with the clinical practice.

Continuous surveillance programs of emergent pathogens and antimicrobial resistance studying clinical isolates representative from different geographic areas are necessary for detecting in real time new pathogens, as well as to characterize molecular mechanisms of resistance. In order to be more effective, surveillance programs should be organized as a multicenter laboratory network connected to the main epidemiological public and private infection control centers, helping the health

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care community to optimize the introduction of effective measures for infection control and prevention.

At the Infectious Diseases Division of the Federal University of São Paulo (UNIFESP), we have organized and/or actively participated in different surveillance programs of bacterial, fungi and viral infections. Three laboratories of our division have been granted international excellence level with important contributions: Special Microbiology Laboratory, Special Micology Laboratory and Retrovirology Laboratory.

SPECIAL MICROBIOLOGY LABORATORY-UNIFESP: SURVEILLANCE PROGRAMS OF NOSOCOMIAL BACTERIAL INFECTIONS

Epidemiology of Nosocomial Infections due to Methicillin-resistant *Staphylococcus aureus*

Created in 1990, The Special Microbiology Laboratory has been part and/or has organized several multicenter surveillance studies of bacterial resistance, including community acquired and nosocomial infections with more than 50,000 bacterial samples collected from different sites of infection and colonization.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been the major nosocomial infections agent since the 80's of the last century. One of the clinical settings with higher risk of acquiring MRSA infections presenting considerable morbidity and mortality rates are represented by the chronic renal patients under peritoneal or hemodialysis. In São Paulo, we evaluated the epidemiology of colonization and infecton by *Staphylococcus aureus* in these particular setting of patients by using molecular typing methods (Pignatari et al. 1990, 1991) contributing for the implementation of nosocomial infection control measures at our university hospital, the Hospital São Paulo (Sesso et al. 1998).

At the beginning of the 90's, after conducting an epidemiological study in 8 hospitals of the city of São Paulo, we detected the dissemination of strains with a specific DNA macrorestriction profile generated by pulsed field gel electrophoresis (PFGE) that we named "SP clone". This clone was further identified as the Brazilian Epidemic Clone (BEC), one of the five global major clones that still occurs nowadays in South America (Sader et al. 1994).

The "BEC clone" was well studied in its microbiology, clinical and epidemiological aspects. We observed a high lethality rate, increasing of costs and time of hospitalization associated with blood stream infections due to this particular pathogen when compared to infections caused by methicillin susceptible strains (Moreira et al. 1998). In addition, we detected in a MRSA-BEC strain a decrease in its phagocytosis and killing by neutrophils and monocytes when compared with the susceptible isolates (Salgado et al. 2004).

Concerns of the scientific and clinical community increased substantially with the likely of dissemination of the BEC clone to the community, particularly among children. A study recently performed with children of the city of Goiania, Brazil, addressed this issue and demonstrated that the dissemination of BEC in this particular population is rare (Lamaro-Cardoso et al. 2007). Lately, investigators performing a molecular characterization of the mec staphylococcal gene cassete (SCCmec) from episodes of community acquired infections found the dissemination of methicillin resistant strains within the community exhibiting highly virulent clones that were not genetically related to the hospital acquired ones, such as the BEC (type III SCCmec) in Brazil. This new phenomenon of dissemination of community-acquired-MRSA is increasing rapidly in the USA and Europe (Stryjewski and Chambers 2008). In order to investigate if this MRSA community acquired infection is occurring in Latin America, we tested a large collection of 6,739 MRSA strains isolated from 1997 to 2006 at clinical centers in Brazil, Chile and Argentina. We observed the introduction of new clones of MRSA, specially the pediatric type IV SCCmec, both at the community and at the hospital. This finding has clinical impact regarding clinical practice once that this new clone is more susceptible to other groups of non beta-lactams antibiotics compared with the BEC clone. In the other hand, they may be more virulent if producing toxins such as the Panton-Valentine leukocidin (S.S. Andrade, unpublished data).

Contributions of the Special Microbiology Laboratory-UNIFESP to the SENTRY: focus in Brazil and Latin America

Considering our expertise in conducting surveillance studies in Brazil, we were invited in 1996 to coordi-

nate the Latin America branch of one of the most important resistance surveillance programs worldwide, the SENTRY program. The goal of this program is to determine geographic and temporal trends in the antimicrobial susceptibility profiles of the main pathogens causing community and nosocomial infections. The bacterial strains are collected prospectively at hospitals and laboratories enrolled as sentinel centers which are organized in an international surveillance network from five continents, with the coordinator center located at the State of Iowa, USA. In Latin America, several centers have participated in this program, including institutions from Mexico, Venezuela, Chile, Colombia and Brazil (São Paulo, Florianópolis, Porto Alegre and Brasília). Several reports of the SENTRY program have been published through more than 10 years of collaboration. In 2004, we reported the *in vitro* activity of several antimicrobial agents against more than 20,000 clinical isolates collected from different medical centers in Latin America (Sader et al. 2004). These data have been used to guide empirical therapy and the local implementation of measures for infection control and prevention. Our data supports the conclusion that antimicrobial resistance of Gram-negative bacteria seems to be higher in Latin America as compared to other regions of the world, particularly North America and Europe. Multi-drug resistance among Gram-negative bacilli was particularly observed in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* strains. For *Pseudomonas aeruginosa*, the Brazilians centers exhibited the highest rates of resistance documented worldwide. In addition, the Brazilian rate of Enterobacteriaceae producing expanded spectrum B lactamase (ESBL) was higher than that documented in any other country. On the other hand, vancomycin-resistant enterococci (VRE) and penicillin-resistant pneumococci are less frequently described in Latin America (Jones 2001, Andrade et al. 2003).

The Sentry program has also provided some data on the evaluation of community acquired infections, such as the surveillance of *E. coli* resistance to the antimicrobial group of quinolones in urinary tract infections. This type of information is relevant for establishing concepts of adequacy of empirical therapy in this particular clinical setting (Andrade et al. 2006, Castanheira et al. 2007).

Contributions of the Special Microbiology Laboratory-UNIFESP to the Characterization of New Mechanism of Gram-negative Resistance to Carbapenens

As previously stated, multidrug resistance among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* strains, particularly to the potent group of beta-lactam antibiotics carbapenems, is rising rapidly in Brazil. Invasive infections caused by the both mentioned species of bacteria are usually only susceptible to the antimicrobial group of polymyxins and are responsible for high morbidity and mortality rates among hospitalized patients (Furtado et al. 2007). The mechanism of resistance to carbapenems was originally described as related to the loss of a membrane porine, impairing the transportation of the drug to its target of action. New mechanisms of resistance have been investigated, including the production of metallo-beta-lactamase coded by a gene carried by integrons, as reported since the 90's in Japan and Italy.

Recently, investigators of our group contributed to the detection and characterization of a new metallo-betalactamase gene bla_{spm} reponsible for the production of an enzyme that inactivates carbapenems (Toleman et al. 2002). This gene is not carried by integrons, but has been detected in plasmid, allowing a more effective horizontal dissemination among strains of *Pseudomonas aeruginosa*. In addition, we observed a clonal dissemination of *Pseudomonas aeruginosa* carrying bla_{spm} in medical centers from Brazil (Gales et al. 2003). The rapid identification of carbapenem resistance in these bacteria, particularly from blood stream cultures, is of paramount importance in terms of clinical care. In this way, we recently published a rapid protocol for detection of metallo-beta-lactamases encoding genes by multiplex real-time PCR (Mendes et al. 2007).

SPECIAL MYCOLOGY LABORATORY-UNIFESP: SURVEILLANCE PROGRAMS OF HEMATOGENOUS INFECTIONS DUE TO *CANDIDA* SPP.

Candidemia is a growing problem in tertiary care hospitals all over the world where it is commonly observed among patients with long-stay hospitalization who have

been exposed to antibiotics, immunosuppressive therapy, parenteral nutrition and other invasive medical procedures. Despite all advances in medical practices, hematogenous candidiais is still considered difficult to diagnose, leads to prolonged hospitalization, causes a mortality rate of around 50% and is a financial burden to health care systems (Colombo et al. 1999, 2006).

The epidemiology of candidemia has been extensively studied in the USA and Europe, but not in most countries of Latin America. Brazil is an exception, with information on the incidence rates of candidemia, risk populations, trends in the species distribution as well as antifungal resistance being recorded over the last ten years (Colombo and Guimaraes 2003, Morgan 2005).

The Burden of Candidemia in Brazilian Tertiary Care Hospitals

Recently, Colombo et al. coordinated 2 large laboratory based surveillance studies to evaluate the epidemiology (including incidence rates), microbiology, treatment and outcome of candidemia in Brazilian tertiary care hospitals. One study enrolled 4 large medical centers in the city of São Paulo, and another one enrolled 11 medical centers in 9 different cities in Brazil. In both studies, epidemiological and clinical data were systematically collected by well trained local investigators who were asked to file up case report forms with the aid of a dictionary of terms. *Candida* isolates collected during the study were all sent to a core laboratory located at the Division of Infectious Diseases – UNIFESP for further identification and antifungal susceptibility testing. Data generated during the two mentioned multicenter laboratory based on surveillance studies showed the incidence rates of candidemia ranging between 1.66 and 2.49/1,000 admissions in Brazilian tertiary care hospitals. These rates are considered 2 to 10 times higher than those documented in the USA and European medical centers (Colombo et al. 2006, 2007).

Although the reasons of a higher incidence of candidemia in Brazil is not completely understood, it could be reasonable to suggest that this fact should be related to differences in resources that are available for medical care and training programs of healthcare workers to assist critically ill patients, as well as a more conservative approach to the administration of empirical antifungal therapies and prophylaxis to patients at high risk for candidemia (Colombo et al. 2007).

In terms of susceptible populations, considering data generated by the mentioned nationwide surveillance of candidemia, 30% of all patients who developed fungaemia in public tertiary care hospitals in Brazil were represented by pediatric patients, a significantly greater proportion as compared to North American and European studies (Hajjeh et al. 2004, Tortorano et al. 2006). In terms of coexisting exposures, most adult patients with candidemia were represented by patients with diabetis and other organ failures who have been exposed to multiple risk factors including antibiotics, parenteral nutrition, central venous catheterization and other invasive medical procedures. It is worth mentioning that, at the time of the diagnosis of candidemia, 46% of the patients were admitted at an intensive care unit, and 39% of all patients had undergone surgery up to 30 days before developing candidemia (Colombo et al. 2006).

Consequences of cutting public funding polices to the health system on the epidemiology of nosocomial rates of bloodstream infections have been scarcely discussed. In order to evaluate if patients treated at hospitals under different levels of financial support exhibit differences in the epidemiology of candidemia, we started a multicenter study to compare 2 public tertiary care hospitals in Brazil with the data generated by 6 private hospitals located in São Paulo (1), Rio de Janeiro (3), Curitiba (1) and Salvador (1). We collected data on the epidemiology (including incidence rates), microbiology, treatment and outcome of candidemia using a web-based case report form, and compared the characteristics of candidemia in patients from public and private hospitals. Preliminary analysis of data collected during this study suggest that incidence rates of candidemia (3.04 *vs.* 1.04/1000 admissions, $p < 0.01$), as well as mortality rates ($53 \times 40\%$, $p = 0.06$), were higher in the 2 public tertiary care hospitals than in the 6 private institutions (A.L. Colombo et al., unpublished data).

Species Distribution of Candida and Antifungal Resistance Related to Episodes of Candidemia

Antifungal resistance surveillance programs have been conducted recently by different groups, and there is

mounting evidence suggesting that the emergence of invasive infections due to *Candida* non-*albicans* (CNA) species resistant to fluconazole may be an increasing problem in several medical centers in the world. Fluconazole resistance rates of *C. glabrata* bloodstream isolates range from 7 to 14% in American hospitals, and from 3.7 to 40% in European hospitals (Chryssanthou 2001, Diekema et al. 2002, Ostrosky-Zeichner et al. 2003, Hajjeh et al. 2004, Cuenca-Estrella et al. 2005, Tortorano et al. 2006).

Unlike the epidemiology of North Hemisphere countries, there is still a predominance of non-*albicans Candida* species in Brazil susceptible to fluconazole represented mostly by *C. parapsilosis* and *C. tropicalis* strains (Colombo et al. 1999, 2006, Antunes et al. 2004, Aquino et al. 2005). According to most series collected in Brazilian medical centers, candidemia due to *C. glabrata* persists at a low frequency accounting for less than 5% of the candidemic episodes. Recently, by analyzing the data related to 1000 episodes of candidemia documented in 4 public medical centers throughout a 9-year period, excluding the rising incidence of *C. parapsilosis* strains from 1995 to 2003 (19% *versus* 25%, $p = 0.03$), there were no changes in the distribution pattern of *Candida* species (da Matta et al. 2007).

In terms of antifungal resistance, most concerns are related to the emergence of non-*Candida albicans* species resistant to fluconazole, particularly *C. glabrata* and *C. krusei*. The Special Mycology Laboratory at the Division of Infectious Diseases-UNIFESP is an active member of the ARTEMIS Global Antifungal Surveillance Program, a worldwide comprehensive surveillance network which uses the CLSI M44-A disk diffusion method with fluconazole discs to capture resistance rates at 134 different laboratories located in 40 countries. Our last report documented the antifungal susceptibility of 205,329 yeast strains against fluconazole tested from June 1997 to December 2005. According to this report, 90.1% of all *Candida* isolates tested were susceptible (S) to fluconazole. On the other hand, fluconazole resistance and dose dependent-susceptibility were observed with 30% and 90%, respectively, of all *C. glabrata* and *C. krusei* strains tested (Diekema et al. 2007, Pfaller et al. 2007). In the last 3 years, we have tested more than 2,000 *Candida* bloodstream isolates from different medical centers as part of 3 different multicenter studies. Overall, we found that fluconazole resistance or dose dependent susceptibility occurs in less than 1% of the *C. albicans*, *C. tropicalis* and *C. parapsilosis* strains related to fungaemia. Nevertheless, 8 to 50% of all *C. glabrata* bloodstream isolates tested were considered susceptible dose dependent or resistant to fluconazole, depending on the period and medical centers evaluated. This finding is similar to data generated by other groups in Brazil (Colombo et al. 2006, 2007, da Matta et al. 2007).

Molecular Mechanisms of Antifungal Resistance

Several groups have attempted to characterize the molecular mechanisms of antifungal resistance with *Candida albicans* and some non-*Candida albicans* strains in Europe and USA. In Latin America, we were the first group to investigate the mechanisms related to azole resistance by testing *Candida* strains isolated from AIDS patients.

In a collaborative study with Professor Gustavo Goldman from the Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, we described the molecular mechanisms of fluconazole-resistance by evaluating *C. albicans* strains isolated from 9 different medical centers. The *C. albicans* isolates used in this study represent a collection of 20 strains which were obtained from AIDS patients with oral or esophageal candidiasis who received fluconazole during a period of 6 to 12 months. The entire open reading frame of the *ERG11* gene encoding lanosterol 14α-demethylase was sequenced from all *C. albicans* isolates. In addition, we evaluated the overexpression of *ERG11* and several genes encoding efflux pumps for azoles by using a quantitative real-time RT-PCR (Goldman et al. 2004).

After screening all resistant *C. albicans* strains, we found the presence of point mutations in the *ERG11* gene, overexpression of *ERG11*, and genes encoding efflux pumps such as CDR and MDR, as measured by quantitative real-time RT-PCR. Several fluconazole-resistant strains had multiple mechanisms of resistance. A total of 4 mutations previously described, Y132F, K143R, E266D, and V437I, were identified among the strains, while some isolates contained more than one mutation (Goldman et al. 2004).

The mechanisms of azole resistance found in the Brazilian collection of *C. albicans* strains resistant to fluconazole are similar to the results described to date by other groups. In the case of *C. albicans* strains, the fluconazole-resistant phenotype is usually secondary to the coexistence of multiple mechanisms of resistance. Of note, new triazoles have been developed, with the aim of avoiding fluconazole cross resistance. However, cross resistance to voriconazole and posaconazole has been extensively reported with *C. glabrata* and *C. albicans* strains (Sanglard et al. 1996, Perea et al. 2001, Sanglard and Odds 2002, Kanafani and Perfect 2008).

THE BURDEN OF SEPSIS IN BRAZIL AND SOME STRATEGIES TO DECREASE THE ASSOCIATED CRUDE MORTALITY

The high incidence of bloodstream infections and sepsis has been continuously evaluated in our Institution by Salomão et al. Between 1985 and 1986, the incidence rate of blood stream infections at the Hospital São Paulo – Universidade Federal de São Paulo, was 21.7 episodes by 1000 admittances, with a crude mortality rate of 33.4% (Salomão et al. 1992, 1993). A new survey performed at the same institution in 2004 demonstrated that the incidence rate of BSI increased to 26.8 per 1000 admissions exhibiting overall mortality rate of 44% (Segovia, Pereira and Salomão, personal communication). The increasing incidence of nosocomial sepsis has been discussed by different groups in the USA, where a large study showed a substantial increase of BSI caused by bacteria and fungi along the last 20 years (Martin et al. 2003).

Considering the lack of sensitivity of blood cultures, it is reasonable to suggest that laboratory based surveillance studies may underestimate the true incidence rates of BSI and sepsis. Recently, a Brazilian multicenter surveillance of sepsis was conducted in 5 intensive care units in São Paulo using as inclusion criteria laboratory and clinical data for case definition. This study (BASES – *Brazilian Sepsis Epidemiological Study*) was conducted by Silva et al. between may 2001 and January 2002, obtaining incidence artes of 57.9 episodes of sepsis per 1000 pacients-day, or 30.5 episodes per 100 admissions. The crude mortality rates of patients with sepsis, severe sepsis and septic shock were 34.7%, 47.3% and 52.2%, respectively (Silva et al. 2004).

The burden of sepsis in the USA was evaluated by a population-based multicenter surveillance study conducted by Martin et al. (2003) including the evaluation of all hospital admissions between 1979 and 2000. During this study, it was possible to conclude that incidence rates of sepsis in the USA raised from 82.7 episodes/100,000 inhabitants in 1979 to 240.4 episodes/100,000 habitants in 2000 (Martin et al. 2003).

In order to decrease the crude mortality of sepsis, including the concerns with multiresistant pathogens, we have targeted to main aspects: the challenge to use appropriate antimicrobial therapy, and the use of strategies to prevent blood stream infections associated with central venous catheter.

The impact of appropriate therapy in the outcome of BSI was clearly demonstrated some decades ago. We have found that appropriateness of antimicrobial therapy, which happen when the bacteria is susceptible to the antimicrobial administered, has a profound impact on survival rates of patients with blood stream infection and sepsis. Overall mortality was 33.4% and it could be as low as 21% in patients receiving appropriate therapy, and as high as 57.1% in those receiving inappropriate therapy. Interestingly, patients who received inappropriate therapy and were administered appropriate therapy thereafter based on cultures results or lack of clinical responses had intermediate mortality (34.1%). However, changing antibiotics in patients already with septic shock did not improve outcomes (Salomão et al. 1993). There is increasing evidence that the delay in initiating the antimicrobial therapy has profound impact in mortality. Kumar et al. showed that the duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock (Kumar et al. 2006).

A relevant aspect to be mentioned and monitored is that nosocomial acquired primary bloodstream infections are mainly associated with central venous catheter (CVC-BSI). In a multicenter study, we found increased device-associated nosocomial infections, including CVC-BSI, in Intensive Care Units from developing countries, as compared to the USA and Europe (Rosenthal et al. 2006). We succeed to reduce the rates

of central vascular catheter-associated bloodstream infections in an adult intensive care unit by using education and performance feedback (Salomão et al. 2005) and reached infections rates similar to the developed countries by switching from an open intravenous infusion system to a closed system (Salomão et al. 2006).

RETROVIROLOGY LABORATORY-UNIFESP: SURVEILLANCE PROGRAMS OF HIV-1 ESCAPE AND RESISTANCE

Viruses are ubiquitous and the most prevalent biological entity on the planet (Angly et al. 2006, Koonin et al. 2006). However, viruses have constantly to adapt to selective pressures offered by the environment where they propagate. Some of these pressures are not present in nature, such as the pressure exerted by antiretroviral drugs, while others are represented by the host immune response (Bailey et al. 2004). Viruses employ many strategies to escape host defenses or other pressures. Viral evasion strategies enable the virus to avoid recognition by the immune response by changing its epitopes, to interfere with cellular immune responses by disabling peptide presentation, to interfere with immune function by secreting viral encoded cytokines or blocking apoptosis, and to interfere with antiviral drug activity by incorporating drug resistance mutations (Domingo 1997, Vossen et al. 2002, Alcami 2003, Peterlin and Trono 2003, Clavel and Hance 2004, Leslie et al. 2004, Smith 2004, Manrubia et al. 2005, Telenti 2005, Martinez-Picado et al. 2006, Schneidewind et al. 2007). Sophisticated DNA viruses have a greater genetic background that allows them to make use of different escape routes, including viral mimicry by incorporation of cellular genes into their genomes (Chaston and Lidbury 2001, Alcami 2003). RNA viruses have smaller genomes and, due to their highly mutation rates, they cannot extend their genomes beyond a limit, being unable to incorporate new and different coding capacities into their genomes (Domingo et al. 1996, 2001, 2005, Domingo 1997, Chaston and Lidbury 2001). RNA virus relay basically on genetic diversification to escape elimination by host defenses or chemical pressures. Diversification gives rise to viral variants with increased resistance. Acquisition of resistance leads to diminished viral fitness (Bleiber et al. 2001, Buckheit 2004, Smith 2004). Viral fitness is generally accepted as the relative ability of a virus to replicate in a defined environment, and is used to describe the viral replication potential in the absence of the selective pressure that originated resistance.

HIV-1 and Genetic Diversity

Global diversity

Almost 30 years since the first cases of the acquired immunodeficiency syndrome (AIDS), our power to manage infected patients or to reach an effective control of the AIDS pandemic remains limited (Rambaut et al. 2004). According to the 2007 UNIAIDS report, there are over 30 million people living with HIV today. Although HIV global prevalence has leveled off, and the number of new infections has decreased, 2.5 million individuals acquired HIV infection and 2.1 million died of AIDS in 2007. AIDS is among the leading causes of death globally and remains the primary cause of death in Africa (www.uniaids.org). HIV strains are divided into two types, HIV-1 and HIV-2. HIV-1 strains are responsible for the global epidemic drive and are classified in Groups, M, N, and O. Group M encompasses 9 genetic subtypes and more than 40 circulating recombinant forms (CRF) (www.hiv.lanl.gov). HIV-1 global distribution is complex and dynamic, with regional epidemics harboring only a subset of the global diversity. HIV-1 strains differ enormously in terms of global prevalence. 6 viral lineages account for the majority of HIV-1 infections: HIV-1 subtypes A, B, C, D, and two of the CRFs, CRF01-AE and CRF02_AG, respectively. Many of the known subtypes and recombinant forms are currently rare in the epidemic. Groups O and N are rare in the pandemic (McCutchan et al. 2004, Kijak and McCutchan 2005, Sa Filho et al. 2005, De Sa Filho et al. 2006, McCutchan 2006, Sanabani et al. 2006, Taylor et al. 2008).

HIV-1 Subtypes in Brazil

The HIV-1 Brazilian epidemic shows a heterogeneous distribution of HIV-1 subtypes and recombinants (Sa Filho et al. 2005, De Sa Filho et al. 2006). The most prevalent strains belong to subtype B and were the first introduced in the country in the 1970s. Subtype F strains

appeared in the 1980s and always accounted for not much than 10% of all infections. More recently, BF recombinants are gaining ground even surpassing the number of subtype F infections. Subtype C strains result from a later introduction and are prevalent in Southern Brazil. Besides plain subtypes, a number of CRFs, have already been identified in Brazil, but their impact on the epidemic is still not clarified (www.hiv.lanl.gov).

Intrahost Viral Diversity

In an individual, HIV-1 maintains a rapid evolution pace resulting from high mutation rates, large population sizes, accelerated replication dynamics, and high recombination frequencies. As a result, HIV populations correspond to a swarm of mutants harboring different mutations, and can be referred to as a quasispecies (Domingo 1985). Quasispecies arise from a balance between mutation and natural selection, originating a population of variant viruses. Viruses in a quasispecies cannot be seen as independent entities, but as linked by mutational couplings, with the entire distribution forming a cooperative community that evolves as a single unit (Duarte et al. 1994, Domingo et al. 1996, 1998, 2001, 2005, 2006, Domingo 1997, Holmes and Moya 2002, Jenkins et al. 2002). In a quasispecies, natural selection is no longer directed toward the single fittest variant, but instead acts on the whole mutant distribution. Viral mutant distributions may promptly adapt to new selective pressures due to high mutation rates.

Escape and Resistance

Escape

The genetic background of different individuals influences their susceptibility to HIV-1 infection and disease progression. Differences on viral susceptibility have been attributed to specific HLA types, chemokines and chemokine receptor polymorphism. More recently, 2 cellular proteins, APOBEC3G and TRIM5, have been described as additional antiviral components of the innate immunity (Sheehy et al. 2002) HIV-1 has evolved strategies against the action of both proteins.

Antibodies are important elements of the immune response and succeed in controlling several viral infections. However, HIV-1 is able to replicate continuously in the presence of a vigorous antibody response. The HIV-1 genome encodes two envelope glycoproteins: the transmembrane gp41 anchors the Env complex in the viral envelope and mediates fusion with the host cell membrane, while the surface gp120 interacts with cellular receptors. HIV-1 gp120 is heavily glycosylated and contains 5 "hypervariable" domains, V1 to V5. HIV-1 envelope proteins avoid neutralizing antibody by a number of different strategies. Mechanisms of humoral escape include: hiding of critical neutralizing epitopes, extension of gp120 variable loops, conformational protection of viral receptor binding motifs, and extensive glycosylation of envelope surface proteins. It has been demonstrated that acutely infected patients produce NAbs against autologous virus within months of seroconversion (Albert et al. 1990, Tremblay and Wainberg 1990, Richman et al. 2003, Wei et al. 2003, Frost et al. 2005), but although the early produced NAbs reach fairly high titers, escape mutants are rapidly selected due to the ongoing viral replication (Richman et al. 2003, Wei et al. 2003). It also has been shown that chronically HIV-1 infected patients develop NAbs against earlier viral isolates, but they fail to neutralize contemporaneous virus (Albert et al. 1990, Tremblay and Wainberg 1990, Skrabal et al. 2005).

Cytotoxic T-lymphocytes (CTL) are important against HIV-1 (Smith 2004, Betts et al. 2006). CTL destroys HIV infected cells upon recognizing viral peptides bound to class I major histocompatibility complex (MHC) molecules. Different MHC alleles bind viral peptides with distinct affinities. MHC alleles, which bind particular viral peptides strongly, often result in vigorous cellular responses against those viral epitopes. Avoidance of cellular responses results in replicative advantages. Evasion of CTL targeting involves mutations within and around recognized epitopes, and results in the lack of peptide binding to the Class I MHC grove, or non-recognition by the CTL T cell receptor, or interference with peptide processing (Phillips et al. 1991, Goulder et al. 1997, Price et al. 1997, Smith 2004). CTL escape mutations are associated with increased viral loads and rapid disease progression (Koenig et al. 1995, Borrow et al. 1997, Goulder et al. 1997). However, mutations associated with CTL escape cause significant viral fitness costs. Fitness costs correlated with CTL

evasion have been demonstrated in patients carrying the B*57, B*5801, and B*27 HLA alleles (Friedrich et al. 2004, Smith 2004, Martinez-Picado et al. 2006, Crawford et al. 2007).

Resistance

Several classes of drugs are used against HIV-1 and target different steps of the viral replication cycle. They include: nucleoside analogues, which act as DNA synthesis terminators inhibiting reverse transcription; nonnucleoside reverse-transcriptase antagonists, which directly inhibit reverse transcriptase; protease inhibitors interfering with viral maturation; entry inhibitors including CCR5 blocking agents and fusion inhibitors, designed to halt HI1 penetration into permissible cells; and the newly approved integrase inhibitor that acts by blocking HIV-1 integration into the cellular genome (Lucas et al. 1999, Dybul et al. 2002, Kress 2004, Kuritzkes 2004, Luber 2005, Piacenti 2006, Evering and Markowitz 2007, Opar 2007, Hendrickson et al. 2008, Lagnese and Daar 2008, Laurence 2008, Strizki 2008). Antiretroviral drugs are used in different combinations, usually of 3 antiretroviral drugs, with 2 nucleoside analogues and either a protease or a nonnucleoside inhibitor, with regimens starting to feature entry inhibitors when HIV infection has not been controlled by other drug combinations (http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed April 19, 2008).

The simultaneous use of different drugs is known as highly active antiretroviral therapy (HAART). The goal of HAART is to achieve and maintain viral loads below 50 copies/mL. Suppression below this level is associated with treatment success, helps immunological restoration, and difficults the onset of viral resistance. The development of drug resistance requires the concurrence of 2 factors: antiretroviral drug exposure and ongoing viral replication. Initially thought as a cure for HIV infection (Perelson et al. 1996), today we know that HAART cannot eradicate HIV-1 (Wong et al. 1997, Siliciano 1999, Siliciano and Siliciano 2000, Bailey et al. 2006). Undoubtedly, HAART has increased the quality of life for HIV-1 infected persons. However, limitations of HAART started to appear by the identification of latent HIV-1 reservoirs (Blankson et al. 1999, 2002, Siliciano 1999, Siliciano and Siliciano 2000, 2004, Bailey et al. 2006). These reservoirs act as viral archives ready to replenish the pool of replicating virus. Although drug therapy is efficient at controlling plasma viral levels, viruses in reservoirs are not targeted by the drugs. Because viral recombination was not taken into account, resistance mutations were thought to accumulate in distinct genomes in an ordered fashion (Molla et al. 1996). Recombination allows the virus to exchange resistance mutations in a nonlinear fashion, leading to rapid evolution leaps of resistant mutants, even from different reservoirs (Morris et al. 1999, Zhuang et al. 2002, Levy et al. 2004, Charpentier et al. 2006, Nora et al. 2007). Finally, seminal studies from patients undergoing HAART wrongly concluded that the virus was not experiencing evolution if viral loads were undetectable (Wong et al. 1997, Finzi and Siliciano 1998). Although not detectable in the plasma, ongoing viral replication in localized niches would inevitably lead to the incorporation of mutations, and some would result in virus with diminished drug susceptibility (Chun et al. 1999, Martinez-Picado et al. 2000). Evolution of drug resistance reduces our ability to control HIV-1. Drug therapy failure results from the accumulation of drug resistance mutations. The genetic barrier for resistance varies from drug to drug. Resistance to some drugs, as nonnucleoside inhibitors, may happen after just a brief exposure to the drug. Conversely, resistance to protease or nucleoside inhibitors generally requires the selection of a combination of mutations (Clavel and Hance 2004, Nettles et al. 2004, Lucas 2005, Wood et al. 2005, Taylor et al. 2008). Mutation in both protease and reverse transcriptase may lead to a reduction in viral fitness, with either enzyme displaying a profound impairment (Bleiber et al. 2001, Buckheit 2004). Resistance to HIV-1 fusion inhibitors is also associated with impaired fitness, while resistance to nonnucleoside reverse transcriptase inhibitors has a less evident effect on viral replication (Schmit et al. 1996, Chen et al. 2005). Acquisition of viral drug resistance generally follows a common series of events: the initial selection of drug-resistance mutations causing viral fitness reductions and, subsequently, the introduction of compensatory mutations enabling the virus to recover fitness (Bleiber et al. 2001, Buckheit 2004).

Drug-resistant mutations are detected in drug-naïve patients (Ribeiro et al. 1998, Brumme et al. 2007,

Smith et al. 2007, Sucupira et al. 2007, Little et al. 2008), but recent data suggest that the prevalence of drug-resistant HIV in newly diagnosed patients may have leveled off at approximately 10% over the past several years (Kuritzkes 2007). However, existence of HIV drug resistance prior to the initiation of antiretroviral therapy can be a major impediment to successful treatment outcomes (Ribeiro et al. 1998, Daar 2007, Metzner et al. 2007). Data on resistance patterns for new drugs and new drug classes are beginning to emerge. All 3 new categories of antiretroviral agents (chemokine receptor inhibitors, second-generation non-nucleosides, and integrase inhibitors) have shown vulnerability to drug resistance (Kuritzkes 2007). Moreover, minority HIV-1 variants that may not be detected by current resistance tests may have an impact on treatment, limiting future therapeutic approaches (Metzner 2006, Daar 2007).

HIV-1 Resistance in Brazil

In 1996 the Brazilian Government approved a Federal law that established an universal access to HAART for citizens living with HIV-1 (www.aids.gov.br). There are approximately 185,000 HIV-1 infected individuals receiving HAART today in Brazil (www.aids.gov.br). Although introduction of HAART has certainly slowed the disease progression among infected people, some of its consequences are the emergence of viral resistance and the transmission of resistant strains. HIV-1 resistance mutations, to at least one class of drugs, can be detected in a high percentage of the treatment experienced by Brazilian individuals (Cavalcanti et al. 2007, De Sa Filho et al. 2008), but the levels of transmitted resistance are comparable to the ones observed in Europe and in the United States (Brindeiro et al. 2003). However, 2 studies indicated much higher primary resistance levels in the coastal cities of Santos and Salvador (Pedroso et al. 2007, Sucupira et al. 2007). Because Brazil was a pioneer in Government sponsored broad drug distribution, there is a constant need to survey HIV-1 resistance levels in treatment naïve and experienced individuals living in the country.

Future perspectives

We are currently conducting a new nationwide surveillance program of nosocomial blood stream infections at 15 medical centers located in different regions of Brazil named SCOPE Brazil (Surveillance and Control of Pathogens of Epidemiological Importance). This program will combine for the first time the efforts of all investigators of our Divison interested in the evaluation of nosocomial bloodstream infections, regardless of the pathogen that causes the disease. We believe that this is a compreensive and effective program that will contribute to more adequate infection control and antimicrobial use of policies in Brazil.

In addition, with the collaboration of Marcio Nucci from the Federal University of Rio de Janeiro, we are establishing the Latin American Candidemia Network, a group of 12 investigators that will conduct a laboratory based surveillance study of candidemia involving 24 medical centers within 9 different countries in Latina America.

With respect to HIV infection, considering the universal access of anti-retroviral drugs in Brazil, it is mandatory to constantly monitor HIV resistance in our population. Our Division is currently involved in checking resistance levels in groups of recently infected patients living in different areas of the country. We have a number of ongoing countrywide collaborations that allow us to collect samples from various locations. Studies like this one are helping us to gauge the primary rate of HIV resistance in Brazil. We are also addressing the stability of drug resistance mutations in naïve patients that acquired the virus from drug experienced patients. Furthermore, we are conducting a series of studies on APOBEC3s polymorphisms encountered in the infected Brazilian population, and investigating how they influence the levels of hypermutation on the viral genome. Finally, we are also constantly surveying the HIV-1 subtype distribution in the country monitoring HIV-1 propagation in Brazil. We were the first group identifying not only one, but two HIV-1 Circulating Recombinant Forms in the Brazilian epidemic.

RESUMO

Várias alterações epidemiológicas ocorreram no perfil das doenças infecciosas hospitalares e comunitárias nos últimos 25 anos. Mudanças sociais e demográficas possivelmente relacionadas com esse fenômeno incluem o rápido crescimento populacional, o aumento da migração urbana e deslocamento

através de fronteiras internacionais por turistas e imigrantes, alterações nos habitats de animais e artrópodes que transmitem doença assim como o aumento no número de pacientes com deficiências nas respostas de defesa. Os programas contínuos de vigilância de patógenos emergentes e resistência antimicrobiana são necessários para a detecção em tempo real de novos patógenos assim como para caracterizar mecanismos moleculares de resistência. Para serem mais efetivos, os programas de vigilância dos patógenos emergentes devem ser organizados em uma rede de laboratórios multicêntricos ligados aos principais centros de controle de infecções, públicos e privados. Os dados microbiológicos devem ser integrados a guias terapêuticos adaptando práticas terapêuticas à ecologia local e aos padrões de resistência. O artigo apresenta uma revisão dos dados gerados pela Disciplina de Infectologia, Universidade Federal de São Paulo, contemplando sua participação nos diferentes programas de vigilância de doenças infecciosas hospitalares e adquiridas na comunidade.

Palavras-chave: doenças infecciosas emergentes, HIV, AIDS, candidemia, resistência antimicrobiana, bacteremia, sepsia, infecções hospitalares.

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