http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 14 (6): 392-398, 2011
ISSN 1028-8880 / DOI: 10.3923/pjbs.2011.392.398

Characterization of HIV-1 Genotypes and Antiretroviral Drug-resistance Mutations among Patients in Burkina Faso

[1]T.S. Kagone, [1]H. Hien, [1]N. Meda, [1]P.S. Diagbouga, [2]A. Sawadogo, [3]J. Drabo, [4]M. Peeters, [4]L. Vergne, [4]E. Delaporte, [5]C. De Souza, [5]M. Gbeassor and [6]J. Simpore
[1]Centre MURAZ Bobo-Dioulasso, Burkina Faso
[2]CHUSS Bobo-Dioulasso, Burkina Faso
[3]CHUYO Ouagadougou, Burkina Faso
[4]Laboratoire de Rétrovirologie IRD Montpellier, France
[5]Faculté des Sciences Université de Lomé, Togo
[6]Centre de Recherche Biomoléculaire Pietro Annigoni, CERBA Université de Ouagadougou, Burkina Faso

Abstract: The purposes of this study were: (1) to describe the genetic variability of HIV strains found in Burkina Faso, (2) to characterize non-B HIV strains mutation profiles selected by ARVs and (3) to detect possible resistances induced by ARV drugs. From 30 October 2002 to 20 November 2003, 132 HIV1-positive patients taking Highly Active Antiretroviral Therapy (HAART) for more than one year in Bobo-Dioulasso and Ouagadougou were included. T-CD4+ lymphocytes count was done using Dynabeads technique while genotypic test and ARV-resistance tests were conducted using Pol sequencing that codes for reverse transcriptase reverse, integrase and protease. Due to undetectable viremia, 86 samples out of 132 could not be characterized. Whereas in the 46 others that had a viral load exceeding 1000 copies mL^{-1}, the following HIV-1 subtypes were identified: CRF06 (54,55%); CRF02(38,63%); CRF01 (4,55%) and subtype A (2,27%). In addition, several mutations related to PI, NRTI and NNRTI resistance were isolated in 27 samples. This study found a huge genetic HIV-1 polymorphism in Burkina Faso. The level of acquired resistance to ARV after one year of treatment amounted 20.4%. These results clearly show that there is imperative need to set up an ARV resistance surveillance network in Burkina Faso to guide treatment strategies and follow the extension of the phenomenon in the country.

Key words: HIV-1 polymorphism, drug resistance, HAART, mutations, Burkina Faso

INTRODUCTION

Since the discovery of HIV-1 in 1983 (Vahlne, 2009; Miedema, 2008) and of HIV-2 in 1986 (Agrawal *et al*., 2010), the Human Immunodeficiency Viruses, the etiologic agents of the Acquired Immunodeficiency Syndrome (AIDS), kept propagating throughout the world, thus causing a pandemic with tragic consequences. HIV positive people who have resistance to antiretroviral drugs are often co-infected with HHV8 (Ilboudo *et al*., 2009), hepatitis B (Ilboudo *et al*., 2007), Toxoplasma gondii (Ouermi *et al*., 2009), pathogenic bacteria and intestinal parasites (Simpore *et al*., 2009). After 1987, the year when AZT (first antiretroviral) (De Clercq, 2009a; Zhang, 2010) marketing was authorized, many ARVs treating HIV infection were developed. They target various viral enzymes (reverse transcriptase, integrase and protease) and viral co-receptors. Today, these antiretroviral drugs belong to six classes: nucleoside/nucleotide and non-nucleoside reverse transcriptase analogues inhibitors, protease inhibitors, entry and fusion inhibitors and integrase inhibitors (De Clercq, 2009b). With the increasing availability of antiretroviral (ARV) drugs in developing countries, the emergence of ARV-resistant strains represents a major public health stake and its prevention constitutes one of the current treatment recommendations. In this context, the ARV-resistant mutated strains pose not only a direct problem in the management of the concerned patient but also an indirect challenge as they are likely to be transmitted to HIV-negative people. Viral resistance is related to mutations in the pol gene that codes for reverse transcriptase and protease, therefore modifying some amino-acids in these enzymes. Thus, their become

Corresponding Author: Jacques Simpore, Génétique et de Biologie Moléculaires Centre de Recherche Biomoléculaire, Pietro Annigoni, Université de Ouagadougou, 01 BP 364 Ouagadougou 01 Burkina Faso
Tel: +226 50361232, +226 50363242

insensitive to the concerned antiretroviral drugs. The first case of resistance to antiretroviral treatment was announced in 1989 (De Clercq, 2009a). In developing countries, social, economic and health conditions are factors conducive to a poor patient compliance and therefore to potential resistance emergence. Among the problems encountered, there are an anarchic circulation of molecules, problems in drug supply chain, lack of infrastructures, inadequate number of trained personnel, high cost of biological tests required for patient follow-up and the poor compliance of patients taking ARVs. It should be specified that the genetic diversity of HIV, particularly in Africa, is also a potentially important factor leading to natural resistance or emergence of resistance to some ARVs. Therefore, treatment efficacy could be influenced by HIV genetic diversity. Subsequently, HIV-2 and HIV-1 strains of group 0 are naturally resistant to Non-Nucleoside Analogue Reverse Transcriptase Inhibitors (NNRTI) (Wittkop *et al*., 2011). In group M, sub-type F strains are insensitive to NNRTI under trial (Almeida *et al*., 2009) and the sub-type G strains seem to be less sensitive to some protease inhibitors (IP) *in vitro* (Velazquez-Campoy *et al*., 2003). Moreover, mutations in nelfinavir resistance of sub-type G strains do not seem to be similar to those noted in sub-type B (Soares *et al*., 2010). A study carried out in Uganda suggests that sub-type D could more quickly develop a nevirapine resistance than sub-type A, in women receiving a single dose nevirapine to prevent mother-to-child HIV transmission (Eshleman *et al*., 2001). Besides, many minor mutations, in particular in protease gene, were discovered among treatment naive patients infected by non-B (Vergne *et al*., 2000; Simpore *et al*., 2007) HIV strains. These mutations are identified through genotypic tests. Therefore, characterization of HIV-1 strains is currently an irreplaceable test for patients facing therapeutic failures. The goal of this study was: (1) to describe the genetic variability of HIV strains found in Burkina Faso (2) to characterize non-B HIV strains mutation profiles selected by ARVs and (3) to detect possible resistances induced by ARV drugs.

MATERIALS AND METHODS

From October 30, 2002 to November 20, 2003 in Bobo-Dioulasso, Burkina Faso, we conducted a cross-sectional study. This study was done at the inception of the Highly Active Antiretroviral Theray (HAART) programme in Burkina Faso when less than 200 HIV patients were treated. We used Centre MURAZ Virology Laboratory in Bobo-Dioulasso and IRD (Institute of Research for Development) Virology Laboratory, a UNAIDS Collaborative Centre in Montpellier (France).

STUDY POPULATION AND PROCEDURES

The study population comprised HIV-1 patients under ARVs, regularly followed up in Bobo-Dioulasso at Internal Medicine Department, Sourô SANOU University Hospital) and Centre MURAZ and in Ouagadougou (Day Care Hospital, Yalgado OUEDRAOGO University Hospital). In addition to University Hospital, a small number of patients were followed up in various private and public health facilities in Ouagadougou: Saint Camille Medical Centre, Pissy District Hospital, Yentema Private Clinic, Office of the President Health Centre, Clinique de la Paix, Clinique Suka and the Burkinabe Association for Family Welfare (ABBEF) Health centre.

Inclusion criteria: The study involved patients who were taking ARV treatment for at least one year and have given their verbal consent.

Sample collection and processing: The 132 patients had venous blood samples collected in two EDTA tubes for medical tests.

Immunological testing: For the biological monitoring, the count of circulating TCD 4 lymphocytes was realized using Dynabeads technique in Centre MURAZ Microbiology-Immunology Laboratory.

Virological study: The Pol gene was sequenced at the IRD Virology Laboratory in Montpellier (France). For the sequencing, plasma and PBMC were separated by centrifugation on ficoll gradient. ARN was extracted from plasma using Qiamp viral RNA mini kit (QIAGEN France). Reverse transcription was realized using Expand Reverse Transcriptase enzyme (Boehringer Mannheim) and reverse IN3 primer (40 pmoles μL^{-1}) 5'-TCTATBCCATCTAAAAATAGTACTTTCCTGATTCC-3'. A pair of primers, G25REV (5'-GCAAGAGTTTTGGCTG AATGAG-3') and IN3 (5'-TCTATBCCATCTAAAAAT AGTACTTTCCTGATTCC-3') amplified a region of 2400 Pb which was used as matrix for the second amplification, with AV150 (5'-GTGGAAAGGAAGGAC ACCAAATGAAAG-3') and polM4 primers (5'-CTATT AGCTGCCCCATCTACATA-3'). PCRs conditions (1st and 2nd rounds) were 5 min at 92°C (denaturation), followed by 35 cycles at 92°C for 20 sec (denaturation), 50°C for 30 sec (primers pairing), 72°C for 10 min (polymerization) with a final elongation lasting 10 min at 72°C. Amplification was verified on a BET-TBE-agarose gel (1%) at 120 V for 30 mn, with a molecular weight marker. Sequencing was realized using an automatic sequencer Applied Bio System 3100 with 16 capillaries. The Big Dye Terminator kit version 3.1 (Applied

Biosystems) was used and the sequence reaction volume was 20 μL (2 μL of Tris-MgCl2 5X buffer, 1 μL of oligo at 3, 2 pmol μL^{-1}, 2 μL of purified DNA 40 to 100 ng, 4 μL of premix and 11 μL of H_2O). PCR conditions for sequencing were 25 cycles: 96°C for 20 sec for denaturation, 50°C for 30 sec for hybridation, 60°C for 4 min to reinforce Taq incorporation and a temperature drop to 4°C. The proteic sequences coding protease and RT were compared to a B consensus sequence for the detection of major and minor mutations using BETA TEST algorithm (http://hivdb.stanford.edu/). Pol gene phylogeny enables us to determine HIV-1 strains circulating in Burkina Faso.

Data analysis: Characteristics of the study population are described in number and percentages with their confidence intervals for categorical variables and in means and medians with their spread for continuous variables. The number and percentages of genetic polymorphism characterized in this population are reported overall. Resistance mutation profiles were interpreted with French National Agency for AIDS Research (ANRS) and Stanford algorithms. The number and percentage of patients with genotypic resistances detected are reported overall and according to treatment characteristics.

RESULTS AND DISCUSSION

Demographic and clinical characteristics of the study population: The study yielded the following results: the mean age of the 132 patients was 38.15 years±1.42 (15 to 64 years). Women represented 72% of patients with a 95% confidence interval spanned between 63.37 and 79.26. The mean of CD4 was 320 μL^{-1}, range (25-1692). The distribution of the study population, according to Centers of Diseases Control and Prevention classification was as follows: stage A (18.18%), stage B (75.00%) and stage C (6.82%). The mean treatment duration for our HIV-infected patients was 14 months.

Treatment experiences: All patients received HAART in accordance with the first line protocol recommended in Burkina Faso. Hundred percent (100%) of the study population had received an NRTI; 73.5% had received an NNRTI; 28.0% had received a PI while 57.0% (12) of the patients were undergoing their 3rd line therapy and were more exposed to developing resistance mutations. Six patients who had interrupted their treatment presented resistance mutations. Forty patient who were in therapeutic failure had a CD4 rate <200 mm^{-3} after 14 months of treatment. First line treatment was maintained by 82 patients (62.12%): 2 NRTI plus 1 PI or 1 NNRTI. But the other patients (37.88%) had changed and gone through several lines of treatments. The main reasons for these changes in therapeutic lines were related either to poor treatment compliance, to HAART side effects or to therapeutic failures.

Pol gene characterization in study population: As shown in Fig. 1, the phylogenic analysis completed by bootscan analyses revealed genetic polymorphisms in the pol genes of HIV-1 strains found in Burkina Faso. Strains pol gene characterization (n = 44) showed an important genetic diversity of HIV-1 in Burkina Faso. The recombining CRF06 strain was predominant (54.55%). The recombining forms CRF02-AG (38.63%), CRF01-AE (4.55%) and sub-type A (2.27%) co-circulated in the study population.

Emergence of HIV-1 resistances to ARVs: Among the 132 patients, 86 (65.20%), who complied well with the treatment protocol, had undetectable viremia and therefore characterization of their HIV-1 strains was not possible. But 46 samples that had a viral load over 1000 copies mL^{-1} could be characterized and 27 other samples exhibited different mutation profiles associated with high rates of resistance to Protease Inhibitors (PI), to Nucleoside Reverse Transcriptase Inhibitor (NRTI) and to Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI). Figure 2 summarizes HIV-1 resistances to various ARVs detected in present study population.

Genotypic mutations associated with resistance to PI, NRTI and NNRTI: All sequenced samples have been analyzed at the level of possible sites of mutations leading to resistances in the sequence coding protease and reverse transcriptase. Table 1 summarizes the mutations found in the study population.

This study found a huge genetic HIV-1 polymorphism in Burkina Faso dominated by circulating recombinant forms CRF06-cpx (54.5%), CRF02-AG (38.6%) and CRF01-AE (4.6%) (Table 2). The level of acquired resistance to ARVs after one year of treatment amounted 20.4% (27/132). Present results agree with the literature which confirms the circulation of sub-type CRF06 in part of West Africa such Niger, Nigeria, Burkina Faso, Mali, Senegal and Côte d'Ivoire (Montavon *et al*., 2002; Nadembega *et al*., 2006; Simpore *et al*., 2007). The study population comprised a large majority of women (72%) which confirms the higher attendance of health centres by women as well as the feminization phenomenon of HIV. Thus, study presents a clear female predominance among

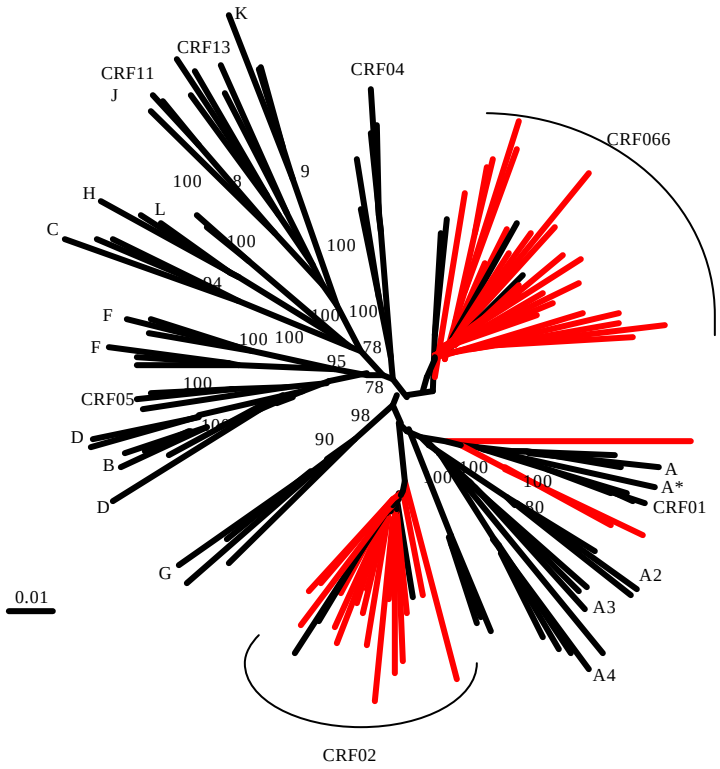

Fig. 1: Radial phylogenic tree of nucleotide sequences of *Pol* gene (Reverse transcriptase, Protease) of reference strains of group M (in black) and of the 44 isolates of Burkina Faso strains from, 2002-2003 (in red). Boostraps (%) reflecting the strength of the branching are noted at the knots of the phylogenic tree

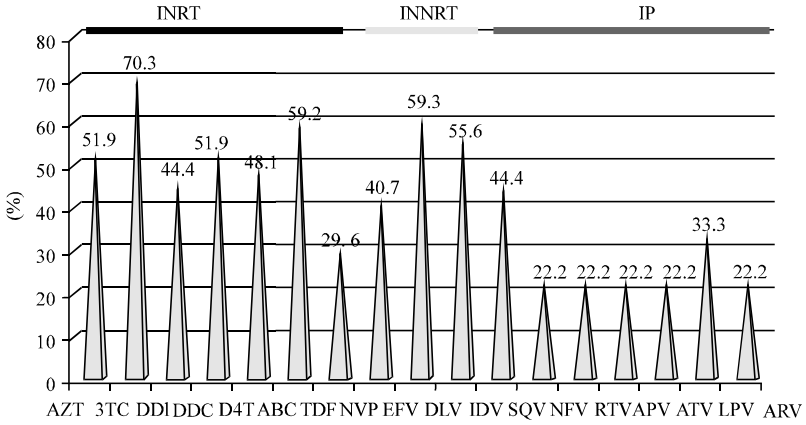

Fig. 2: Percentage of any resistance detected according to ARV drug, Burkina Faso, 2002-2003. Anti-retrovirals used: AZT: Zidovudine; 3TC: Lamivudine; DDI: Didanosine; DDC: Zalcitabine; D4T: Stavudine; ABC: Abacavir; TDF: Ténofovir Disoproxyl Fumarate; NVP: Névirapine; EFV: Efavirenz; DLV: Delavirdine; IDV: Indinavir; SQV: Saquinavir; NFV: Nelfnavir; RTV: Ritonavir; APV: Amprenavir; ATV: Atazanavir; LPV: Lopinavir; INTR: Inhibiteurs Nucléosidiques de la Transcriptase Reverse; INNTI: Inhibiteur Non Nucléosidique de la Transcriptase Reverse; IP: Inhibiteur de la Protéase; ARV: Antiretroviral

Table 1: Number of major and minor mutations inducing resistances to ARVs: PI, NRTI and NNRTI, Burkina Faso, 2002-2003

ARV	Major Mutations								
IP	M46I	I54V	V82A	I84V	L90M	K20I			
No:	4	3	4	3	3	42			
INRT	M184V	Q151M	L74V	V75I	T69A	T215Y	K70R	D67N	
No:	15	2	1	2	7	12	1	1	
INNRT	A98G	K101E	K103N	V108I	Y181C	Y188L	G190A	P225H	
No:	2	2	12	2	1	4	5	1	
ARV	Minor Mutations								
IP	L63P	A71V	G73S	V77I	L10I/V	K20I/V	M36I/R	F53L	L63A
No:	8	2	1	1	9	42	45	1	8
INRT	M41L	E44D	K65R	V118I	F119Y	D67N/D	K70D/R	L210C	K219E	R211K	G333E
No:	9	4	3	3	1	10	6	7	9	3	2
INNRT	V179E	M230L	L234I	F227L	K238N	V106R					
No:	1	2	2	3	2	4					

Table 2: Comparison of strains frequencies in present study and those found by Ouedraogo *et al.* (2003) (18) and Nadembega *et al.* (2006), all in Burkina Faso (19)

Strains	present study n = 46	Ouedraogo *et al.* (2003) n = 70	Nadembega *et al.* (2006) n = 29
CRF06-cpx	54.5%[a]	50.0%[b]	55.2%[c]
CRF02-AG	38.6%[d]	30.0%[e]	31.0%[f]
A1	2.3%[g]	10.0%[h]	6.9%[i]
G		7.1%	3.5%
CRF09-cpx			3.4%
CRF01-AE	4.6%		
Autre		2.9%	
Total	100%	100%	100%

a¬b: p = 0.977 (NS); a¬c: p = 0.639 (NS); b¬c : p = 0.624 (NS) d¬-e: p = 0.635(NS); d¬f: p = 0.919 (NS); e¬f: p = 0.505 (NS) g¬h: p = 0.949 (NS); g¬i: p = 0.917 (NS); h¬i : p = 0.549 (NS) NS: Non-Significant

PLWHIV/AIDS (76.00%), among PLWHIV/ARV (66.23%) and PLWHIV/AIDS/COTI (87.23%) (Montavon *et al.*, 2002; Drabo *et al.*, 2001). In this sense, present findings confirm those of Pignatelli *et al.* (2006) and Drabo *et al.* (2001) who had also found a very high women's attendance of health centres. The study population was relatively young with a mean age of 38.15 years±1.42 and extreme values ranging between 15 and 64 years. This mean age compares with data from a similar study conducted in Côte d'Ivoire (38±5) and Cameroon (Adje *et al.*, 2001). The study population in our study as well as in a similar study conducted in Côte d'Ivoire reflects the population pattern in developing countries that show a mean age below 40 years (Toni *et al.*, 2003). Thus, present findings overlap with UNAIDS findings that revealed that the 20-49 years age group is the largest one among people living with HIV/AIDS (Toni *et al.*, 2003; Delfraissy, 2002; Sagna *et al.*, 2010). After 14 months of treatment, CD4 median in the studied population was at 320 μL^{-1} [25-1692 CD4 μL^{-1}]; which justifies the high rate of resistances (20.45%) found in our study population. However, the average rate of CD4 found in our study was higher than the 118 CD4 μL^{-1} rate found in Cameroon in a similar research with extreme values of 78-167 CD4 μL^{-1} (Adje *et al.*, 2001). These results suggest that the Cameroonian patients were at a more advanced stage of AIDS at the time of the study. The characterization of our study population in clinical stages proposed by CDC shows that only 7% of our study, population was at the advanced C stage.

The recombining CRF06 strain was predominant (54.55%). However, the recombining CRF02-AG (38.63%), CRF01-AE (4.55%) forms and sub-type A (2.27%) also co-circulated in the study population. Present results tally with those found by Ouedraogo-Traore *et al.* (2003) and Nadembega *et al.* (2006) in studies carried out in Burkina Faso (Table 2). In all three studies, no statistically significant differences were found in strains frequencies: CRF06-cpx, CRF02-AG and A1. Among the 46 samples characterized, 27 (58.70%) persons presented resistances to the drugs currently used in triple therapy following a 14-month therapy. These data compare with findings of another study conducted in Abidjan in Côte d'Ivoire in which 57.4% of patients (n = 68) had acquired resistances after ARV (Adje *et al.*, 2001). Nadembega *et al.* (2006) found 37.5% (6/16) resistances in patients under ARV. However, there is no statistically significant difference between the resistance rate we just found and Nadembèga's findings (p = 0.143). Another study was conducted by Vergne *et al.* (2000) in Senegal, where HIV prevalence is very low, detected an 11.8% ARV resistance. Based on these Senegal data, it can be ascertained that the higher the number of HIV-infected people taking HAART, the higher the probability of isolating ARV resistant strains. In our study, twenty-three patients-16 of whom where in therapeutic failure, had a major ARVresistance. These major resistance mutations fell into three classes of ARV: M184V, T215Y, K103N, M46I, K20I, M36I, I84V observed in our study are the major standard mutations found in literature (Adje *et al.*, 2001; Tebit *et al.*, 2008; Varella *et al.*, 2008; Djoko *et al.*, 2011). The antiretroviral drugs helped in achieving a drastic decline in infection morbidity and mortality, turning it into a chronic infection. However, the prescriber and the patient should bear in mind that HIV

infection remains potentially lethal because of the mutations induced by ARV and requires continuous long term treatment to obtain a virological and immunological control.

CONCLUSION

The results obtained from this study suggest that physicians should be more attentive to the monitoring of patients taking HAART, in order to avoid producing and disseminating numerous new resistant strains among their patients. Besides, we believe that HIV vaccine research for Burkina Faso cannot disregard the recombining strains we have just identified, namely: CRF06; CRF02-AG; CRF01-AE 4 and sub-type A 2.

ACKNOWLEDGMENTS

The authors are grateful to the staff of Centre MURAZ Bobo-Dioulasso, CHUSS Bobo-Dioulasso, Laboratoire de rétrovirologie IRD Montpellier, Faculté des sciences Université de Lomé/Togo, Centre Médical Saint Camille and CERBA/LABIOGENE, Université de Ouagadougou. They are deeply grateful to the ANRS (Agence nationale de recherches sur le Sida et les hépatites virales) for the financial support.

REFERENCES

Adje, C., R. Cheingsong, T.H. Roels, C. Maurice and G. Djomand *et al*., 2001. High prevalence of genotypic and phenotypic HIV-1 drug-resistant strains among patients receiving antiretroviral therapy in Abidjan, Cote d'Ivoire. J. Acquir. Immune. Defic. Syndr., 26: 501-506.

Agrawal, S., S. Sawant and J. Shastri, 2010. Prevalence of HIV-2 infection in Mumbai. Ind. J. Dermatol. Venereol. Leprol., 76: 709-710.

Almeida, F.J., E.N. Berezin, R. Rodrigues, M.A. Safadi, M.V. Arnoni, C. Oiveira and L.F. Brigido, 2009. Diversity and prevalence of antiretroviral genotypic resistance mutations among HIV-1-infected children. J. Pediatr. (Rio J.), 85: 104-109.

De Clercq, E., 2009a. Review anti-HIV drugs: 25 Compounds approved within 25 years after the discovery of HIV. Int. J. Antimicrob. Agents, 33: 307-320.

De Clercq, E., 2009b. The history of antiretrovirals: Key discoveries over the past 25 years. Rev. Med. Virol., 19: 287-299.

Delfraissy, J.F., 2002. Patients on Antiretroviral Treatment: Support for People Infected with HIV/AIDS, Recommended by Expert Groups. Flammarion, Paris, France, pp: 42-62.

Djoko, C.F., A.W. Rimoin, N. Vidal, U. Tamoufe and N.D. Wolfe *et al*., 2011. High HIV type 1 group M pol diversity and low rate of antiretroviral resistance mutations among the uniformed services in Kinshasa, Democratic Republic of the Congo. AIDS Res. Hum. Retroviruses, 27: 323-329.

Drabo, J., S. Minougou, M. Ouedraogo and M. Bambara, 2001. ARV treatment: Report of 45 patients followed in Ouagadougou. Proceedings of the 12th International Conference on AIDS and STDs in Africa. Dec. 9-13, Ouagadougou, Burkina Faso, pp: 233-233.

Eshleman, S.H., G. Becker-Pergola, M. Deseyve, L.A. Guay and M. Mracna *et al*., 2001. Impact of human immunodeficiency virus type 1 (HIV-1) subtype on women receiving single-dose nevirapine prophylaxis to prevent HIV-1 vertical transmission (HIV network for prevention trials 012 study). J. Infect. Dis., 184: 941-947.

Ilboudo, D., D. Karou, W.M.C. Nadembega, A. Savadogo and O.D.S. Pignatelli *et al*., 2007. Prevalence of human herpes virus-8 and hepatitis B virus among HIV seropositive pregnant women enrolled in the mother-to-child HIV transmission prevention program at saint Camille medical centre in Burkina Faso. Pak. J. Biol. Sci., 10: 2831-2837.

Ilboudo, D., J. Simpore, D.S. Sanou, D.J. Sia, D. Ouermi *et al*., 2009. Mother-to-child HIV and HHV-8 transmission in neonates at saint camille medical centre in burkina faso. Pak. J. Biol. Sci., 12: 908-913.

Miedema, F., 2008. A brief history of HIV vaccine research: Stepping back to the drawing board?. AIDS., 22: 1699-1703.

Montavon, C., C. Toure-Kane, J.N. Nkengasong, L. Vergne and K. Hertogs *et al*., 2002. CRF06-cpx: A new circulating recombinant from of HIV-1 in west Africa involving subtypes A, G, K and J. J. Acquir. Immune. Defic. Syndr., 29: 522-530.

Nadembega, W.M., S. Giannella, J. Simpore, F. Ceccherini-Silberstein and V. Pietra *et al*., 2006. Characterization of drug-resistance mutations in HIV-1 isolates from non-HAART and HAART treated patients in Burkina Faso. J. Med. Virol., 78: 1385-1391.

Ouedraogo-Traore, R., C. Montavon, T. Sanou, N. Vidal and L. Sangare *et al*., 2003. CRF06-cpx is the predominant HIV-1 variant in AIDS patients from Ouagadougou, the capital city of Burkina Faso. AIDS, 17: 441-444.

Ouermi, D., J. Simpore, A.M.G. Belem, D.S. Sanou and D.S. Karou *et al*., 2009. Co-infection of *Toxoplasma gondii* with HBV in HIV-infected and uninfected pregnant women in Burkina Faso. Pak. J. Biol. Sci., 12: 1188-1193.

Pignatelli, S., J. Simpore, V. Pietra, L. Ouedraogo and G. Conombo *et al.*, 2006. Factors predicting uptake of voluntary counselling and testing in a real-life setting in a mother-and-child center in Ouagadougou, Burkina Faso. Trop. Med. Int. Health, 11: 350-357.

Sagna, T., F. Djigma, M. Zeba, C. Bisseye, S.D. Karou and D. Ouermi *et al.*, 2010. Human papillomaviruses prevalence and genital co-infections in HIV-seropositive women in ouagadougou (burkina faso). Pak. J. Biol. Sci., 13: 951-955.

Simpore, J., V. Pietra, S. Pignatelli, D. Karou and W.M. Nadembega *et al.*, 2007. Effective program against mother-to-child transmission of HIV at Saint Camille Medical Centre in Burkina Faso. J. Med. Virol., 79: 873-879.

Simpore, J., D. Ouermi, D. Ilboudo, A. Kabre and B. Zeba *et al.*, 2009. Aetiology of acute gastro-enteritis in children at saint Camille Medical Centre, Ouagadougou, Burkina Faso. Pak. J. Biol. Sci., 12: 258-263.

Soares, R.O., P.R. Batista, M.G. Costa, L.E. Dardenne and P.G. Pascutti *e al.*, 2010. Understanding the HIV-1 protease nelfinavir resistance mutation D30N in subtypes B and C through molecular dynamics simulations. J. Mol. Graphics Modell., 29: 137-147.

Tebit, D.M., L. Sangare, A. Makamtse, S. Yameogo and H. Somlare *et al.*, 2008. HIV Drug Resistance pattern among HAART-exposed patients with suboptimal virological response in Ouagadougou, Burkina Faso. J. Acquir. Immune. Defic. Syndr., 49: 17-25.

Toni, T.D., P. Recordon-Pinson, A. Minga, D. Ekouevi and D. Bonard *et al.*, 2003. resence of key drug resistance mutations in isolates from untreated patients of Abidjan, Cote d'Ivoire: ANRS 1257 study. AIDS Res. Hum. Retroviruses, 19: 713-717.

Vahlne, A., 2009. A historical reflection on the discovery of human retroviruses. Retrovirology, 6: 40-40.

Varella, R.B., S.B. Ferreira, M.B. Castro, M.D. Tavares and M.G. Zalis, 2008. Prevalence of resistance-associated mutations in human immunodeficiency virus type 1-positive individuals failing HAART in Rio de Janeiro, Brazil. Braz. J. Infectious Dis., 12: 380-384.

Velazquez-Campoy, A., S. Vega, E. Fleming, U. Bacha, Y. Sayed and H.W. Dirr and E. Freire, 2003. Protease inhibition in African subtypes of HIV-1. AIDS Rev., 5: 165-171.

Vergne, L., M. Peeters, E. Mpoudi-Ngole, A. Bourgeois and F. Liegeois *et al.*, 2000. Genetic diversity of protease and reverse transcriptase sequences in non-subtype-B human immunodeficiency virus type-1 strains: Evidence of many minor drug resistance mutations in treatment-naive patients. J. Clin. Microbiol., 38: 3919-3925.

Wittkop, L., H.F. Gunthard, F. de Wolf, D. Dunn and A. Cozzi-Lepri *et al.*, 2011. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): A European multicohort study. Lancet Infect. Dis. 11: 363-371

Zhang, X.Q., 2010. The newest developments in anti-HIV-1 drugs. Yao Xue Xue Bao., 45: 194-204.