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Effects of NAT2 and CYP2C9 Polymorphism in HIV/AIDS Patients on Side effects due to Cotrimoxazole in Yaounde

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A B S T R A C T

Cotri prophylaxis is a simple, inexpensive intervention and is the standard of treatment in developed countries, but there are no clear guidelines for its use in resource-limited countries. CYP2C9 is a polymorphically expressed enzyme responsible for the metabolism of several clinically important drugs, some of which have a low therapeutic index, and N-acetyltransferase (NAT) is a key enzyme in the conjugation of certain drugs and other xenobiotics with an arylamine structure. Studies have shown that genetic predispositions can induce side effects. This is how we envisaged in this work, the study of the polymorphism of the NAT2 and CYP2C9 genes with the aim of establishing a procedure manual for the study of the polymorphism of these genes on the side effects of Cotrimoxazole. The informed consent of 66 patients enabled us to take a blood sample. The samples were placed on filter paper for DNA extraction by the Chelex 100 method at LAPHER-Biotech in Yaoundé. After extraction, the SNPs of the genes were detected by RLFP-PCR. Thus, the enzymes BamHI, KpnI, Taqα-1 for the SNPs of the NAT2 gene and the enzyme BslNI for the CYP2C9 were used for digestion. The present study results showed a participation rate for women well above the rate for men, ie 67.16% against 31.64%. Average age is 43,32±28,28 years. A genotypic predominance of NAT2 * 5/6 was observed, i.e. a frequency of 28.78% (19/66) for NAT2, and CYP2C9 * 3 at 97% (64/66) for Cytochrome P450. Was NAT2 * 4 or 42.42% and a phenotypic predominance of slow acetylators, or 56.71%. Out of 66 patients surveyed 16.7% had side effects, so the occurrence of side effects due to Cotrimoxazole in seropositive patients is not strongly related to polymorphisms of NAT2 (OR = 0.454, P-value = 0.263) and CYP2C9 (OR = 0.74, and P-value = 0.38) in slow acetethylators in this study.

Introduction

HIV is a retrovirus that attacks the immune system and destroys it or renders it ineffective (WHO 2016). Worldwide, there are approximately 38.0 million people living with HIV and 690 thousand deaths linked to opportunistic infections (UNAIDS, 2019). The WHO African Region, where 25.6 million people were living

with HIV in 2019, is the most affected region (UNAIDS, 2019). Cameroon, a country in a generalized epidemic with an HIV prevalence of 3.1% among adults aged 15-49 (UNAIDS, 2019). The burdens of this pandemic are enormous. In the early stages of infection, the subject does not show symptoms. However, the progression of the infection leads to a weakening of the immune system and increased vulnerability to opportunistic infections.

The most advanced stage of HIV infection is AIDS, which can take 10 to 15 years, sometimes longer (WHO, 2016). Currently, there is no cure, but ART can slow the progression of the disease by preventing the virus from reproducing and therefore reducing the amount of virus in the blood of the infected person (called "viral load"). Untreated, the majority of people infected with HIV will show signs of HIV illness within 5 to 10 years, sometimes less (WHO, 2014). AIDS-related deaths were reduced in 2019, 690,000 [500,000–970,000] people died from AIDS-related illnesses globally, compared to 1.7 million [1.3 million – 2.4 million] in 2004 and 1.2 million [860,000 – 1.6 million] in 2010 (UNAIDS, 2021). Although much effort has been made, HIV/AIDS still remains one of the major public health problems in the world, particularly in low- and middle-income countries like Cameroon. Thanks to recent advances in access to antiretroviral treatment and prophylaxis with Cotrimoxazole, HIV-positive people are now living longer and healthier lives. Cotrimoxazole prophylaxis is a simple, well-tolerated and cost-effective intervention for people living with HIV. It should be continued at the start of antiretroviral treatment until signs of immune recovery appear (WHO, 2006). It reduces morbidity and mortality due to opportunistic infections and is therefore recommended for all children exposed to HIV, born to mothers living with HIV (Harouna *et al.,* 2015). Enzymes such as cytochrome P450 are involved in the metabolism, for the bioactivation of sulfamethoxazole to sulfamethoxazole-hydroxylamine, then subsequently, N-acetyltransferase 2 (NAT2) to N-acetoxy-sulfamethoxazole is involved in the metabolism of Cotrimoxazole (Harouna *et al.,* 2015; Butcher *et al.,* 2002). Mutations in the genes encoding NAT2 and CYP2C9 enzymes can alter the metabolic rates of these enzymes and thus become rapid or slow metabolizers (Butcher *et al.,* 2002). It was observed in a study that there were genetic polymorphisms for allelic variants in the CYP2C9 and NAT2 genes in HIV-positive patients that induce the adverse effects (Pirmohamed *et al.,* 2001). Another study showed that the occurrence of adverse effects due to Cotrimoxazole is closely linked to the genotype coding for slow acetylation in the NAT2 gene (Zielinska *et al.,* 1999). Thus, this study conducted in Yaoundé aims to develop a procedural manual for the study of NAT2 and CYP2C9 polymorphisms in relation to side effects in HIV-positive patients taking Cotrimoxazole in Yaoundé.

Hypothesis

The polymorphism of the NAT2 and CYP2C9 genes will not influence the appearance of side effects in HIV/AIDS-positive patients receiving treatment with Cotrimoxazole?

General objectives

The general objective of this work is to develop a procedural manual for the study of NAT2 and CYP2C9 polymorphisms in relation to side effects in HIV-positive patients taking Cotrimoxazole in Yaoundé.

Specific objectives

More specifically, it would be:

-Determine the frequency of NAT2 and CYP2C9 gene mutation in HIV-positive patients by RFLP-PCR;
-Determine the relationship between NAT2 and CYP2C9 polymorphisms in patients taking Cotrimoxazole in Yaoundé;
-Develop a procedure manual to standardize the care of PLHIV/AIDS.

Molecular Analysis

Extraction of DNA

DNA is extracted using the Chelex-100 method (Chelex= chelating Ion Exchange Resin). Chelex-100 or Chelex resin is a specialized resin that chelates metal ions as well as other contaminants.

The filter papers (blotting papers) on which the blood spots were deposited were used for DNA extraction by the Chelex 100 method as described by Plowe *et al.,* (1995). Indeed, the filter papers containing blood spots were excised with a pair of sterile surgical scissors and the pieces obtained were each introduced into sterile Eppendorf tubes and in each tube, 1 ml of saponin (5%) was added. The tubes were after several inversions

incubated at 4° C overnight. After incubation, the tubes were removed and emptied of their liquid content. The filter paper pieces were replaced in other Eppendorf tubes where 1 ml of saponin (5%) was added followed by incubation for 15-30 minutes. After this second incubation period, the filter papers were removed from the tubes and introduced into other 1.5 ml tubes containing 50 µl of 20% chelex stock solution and 150 µl of double demineralized water (DNase free water) heated to 100 °C on the heating block. The tubes were vigorously vortexed for 30 seconds and placed back on the heating block at 100 °C for 10 minutes and then vortexed again during incubation and after incubation. The tubes were centrifuged at 10,000 rpm for 2 minutes followed by a transfer of each of their contents into new tubes which were in turn centrifuged at 10,000 rpm for 2 minutes. The last transfer of the contents of the tubes into new tubes was done very carefully to avoid the transfer of chelex matrix. The last tubes containing the DNA solution were stored at -20°C for the next molecular analyses by PCR.

PCR amplification of the NAT2 gene

The protocol described by Kengne *et al.,* (2016) was used for the amplification of the NAT2 gene using the primers NAT2F and NAT2R (Table I). Each PCR was performed in a total volume of 50 µl and the reaction medium was composed of PCR water, buffer (10X thermopol buffer), 10 mM dNTPs (400 uM of each deoxyribonucleotide), 0.4 µM of each primer, 2.5 U/µl of Taq DNA polymerase and 6 µl of DNA extract. The amplification program previously recorded in the thermal cycler (T3 thermal cycler (Biometra, UK)) was carried out as follows: the initial denaturation is carried out at 95°C for 5 minutes followed by 30 amplification cycles, each cycle of which is a succession of the steps of denaturation at 95°C for 50 seconds, annealing at 55°C for 50 seconds and extension at 72°C for 50 seconds. The final extension at the end of these cycles is carried out at 72°C for 5 minutes. At the end of the amplifications, the PCR products when not directly used can be stored in the thermal cycler at 4°C for 48 hours.

Amplification of the CYP2C9 gene by PCR

CYP2C9 gene amplification was performed according to ZhiYu *et al.,* (2014) using CYP2C9F and CYP2C9R primers (Table I). Each PCR was performed in a total volume of 25µl and the reaction medium consisted of PCR water, buffer (10X thermopol buffer), 10mM DNTP (200 µM of each deoxyribonucleotide), 0.8 µM of each primer, 1.25U/µl of Taq DNA polymerase and 3µl of DNA extract. The amplification program previously recorded in the thermal cycler (T3 thermal cycler (Biometra, UK)) was as follows: the initial denaturation was carried out at 94 °C for 5 minutes followed by 30 amplification cycles, each cycle of which is a succession of denaturation at 94 °C for 30 seconds, annealing at 64.5 °C for 30s, elongation at 72 °C for 30 seconds. The final extension which occurs at the end of these cycles is carried out at 72 °C for 10 minutes. At the end of the amplification, the PCR products when not used directly can be stored in the thermal cycler at 4 °C for 48 hours.

RFLP digestion of PCR products of the NAT-2 gene

Digestion of the 535 bp *NAT2* gene was performed using the method adapted from previously described protocols (Kengne *et al.,* 2016). We used the restriction enzymes Bam HI, KPn I and Taq α-1 (Table II). For each restriction enzyme, digestion was performed in a volume of 20 µl containing PCR water, buffer (10X buffer), 2 ng/µl of BSA, 8 U/µl of restriction enzyme (Bam HI, KPn-I or Taq α-1) and 8 µl of *NAT2* amplicon. The mixes were then incubated at 37°C for the restriction enzymes Bam HI and KPn-1 for at least 16 hours (Overnight) and at 65°C for the restriction enzyme Taq α-1 for at least 6 hours.

RFLP digestion of CYP2C9 gene PCR products

The restriction enzyme BslNI was used for digestion of the *CYP2C9 gene* (Table II) according to a protocol described (ZhiYu *et al.,* 2014). Digestion was performed in a volume of 20 µl containing PCR water, buffer (10X buffer), 4 U/µl of restriction enzyme BslNI and 8 µl of *CYP2C9* amplicon. Digestion was performed in an incubator at 60 °C for at least 6 hours.

Determination of the size of DNA fragments

For the determination of the size of DNA fragments we used agarose gel electrophoresis which is based on the migration of linear DNA molecules through a gel subjected to the influence of an electric field, inversely proportional to the molecular weight of the DNA.

For the NAT2 and *CYP2C9* genes, PCR and RFLP amplification products were analyzed using a mini-gel. The gel solution was cast on an electrophoresis plate with

combs and allowed to solidify for 45 min. Subsequently, after cooling for 15 to 20 min, the gel was inserted into an electrophoretic tank containing a sufficient amount of running buffer (1X TBE). The combs were removed very carefully, revealing wells. Using a micropipette adjusted to 10 µl, the PCR or RFLP products were dissolved in 2.5 µl of loading buffer. After mixing completely, the mixtures were introduced into the wells.

In the first well were introduced molecular weight markers (50 or 100 bp). The electrophoresis tank was then closed and connected to a direct current generator (Fisher Biotech) set at 70 V. The migration was then followed for 45 min using the displacement of bromophenol blue and xylene cyanol. After migration, the gel was then visualized on a high-performance Trans UV illuminator and the pictures were taken.

Statistical Analysis

For the statistical analysis of our data, we used Excel software to calculate the means and standard deviations of demographic variables. For the calculation of the allele frequencies of the *NAT2* and *CYP2C9* genes, the counting method was used. In this method, the frequency of an allele corresponds to the ratio of the number of this allele to the total number of alleles occupying a given locus, i.e. 2N for a sample of N diploid individuals: fqx = (2Nx + Nxy)/2N (with Nx: the number of individuals with phenotype x carrying two alleles x; Nxy: the number of individuals with phenotype xy carrying one allele x and one allele y and N: the total number of individuals studied). For the correlation tests between the phenotype of the *NAT2* and *CYP2C9* genes, NAT2 and side effects, CYP2C9 and side effects; NAT2, CYP2C9 and side effects, SPSS software version 25.0 (SPSS Inc., USA) was used. Odds ratios (ORs) and 95% confidence intervals (CIs) were also determined and a P-value < 0.05 was considered statistically significant.

Results and Discussion

Sociodemographic characteristics

The mean age was 43.32 years ± standard deviation of 28.28 years in a study population of 66 recruited HIV-positive patients; women were predominantly representative with a participation rate of 67.16%; patients on Cotrimoxazole who had side effects represented 16.41% (Table III).

Frequency of side effects of Cotrimoxazole

In our study, we note that few patients developed side effects, around 16.41%, compared to 82.08% of patients who did not have side effects.

Result of NAT2 gene amplification

The electropherogram of the PCR products of the NAT2 gene shows bands at 535 bp indicating the presence of this desired gene in all the patients studied (Figure: 10).

Result of NAT2 gene digestion

At the end of the digestion, the following results observed:

- bands at 535 and 428 bp, 483 bp, 205, 170 and 160 bp, characteristic of (NAT2*4/7) with the enzymes BamHI 3, KpnI 4, Taqα-1 5;
- bands at 535 and 428 bp, 483 bp and 330 and 205 bp characteristic of (NAT2*6/7) with the enzymes BamHI 6, KpnI 7, Taqα-1 8;
- bands at 535 and 428 bp, 483 bp and 330 and 205 bp characteristic of (NAT2*6/7) with the enzymes BamHI 9, KpnI 10, Taqα-1 11;
- bands at 535 and 428 bp, 535 bp and 205, 170 and 160 bp (NAT2*5/7) with the enzymes BamHI 12, KpnI 13, Taqα-1 14 (Figure 12).

Allelic frequency of the NAT2 gene

We determined 3 alleles in our study population with the predominance of the NAT2*4 allele, i.e. a frequency of 42.42% (Figure 14).

Phenotypic frequency of the NAT2 gene

The analysis of the metabolic status of our patients allowed us to determine the slow, fast and intermediate acetylators with the predominance of slow acetylators, i.e. 56.71%, 25.37% of intermediates and 16.41% of fast ones (Figure 16).

Result of CYP2C9 gene amplification

The electropherogram of the CYP2C9 gene amplification products showed bands at 202 bp (as expected) for all samples studied, characteristic of the presence of the CYP2C9 gene (Figure 17).

Result of enzymatic digestion of the CYP2C9 gene

At the end of the digestion we observed bands at 127 and 202 bp. The samples which show bands at 127 bp carry the CYP2C9* 3 genotype, corresponding to the wild phenotype and those which show bands at 105, 75 and 22 bp carry the CYP2C9* 2 genotype corresponding to the phenotype of intermediate metabolizers (Figure: 17).

Phenotypic/genotypic frequency of the CYP2C9*3 gene

In our study, we obtained the phenotypes of intermediate and rapid metabolizers, with a predominance of intermediates, i.e. a frequency of 96.96% (64/66) and an absence of slow metabolizers (0%) (Figure 19).

Result of correlations between NAT2 and the side effects of Cotrimoxazole

The table below shows a low rate of side effects, i.e. a frequency of 16.7% (11/66) compared to 83.3% (55/66) of the frequency of patients who did not have side effects. However, slow acethylators are more likely to have cotri side effects than slow acethylators with an OR=0.454, P-value=0.263, the association is not significant between the NAT2 gene and the side effects due with Cotrimoxazole (Table: IV).

Results of correlations between CYP2C9 and side effects of Cotrimoxazole

The table below shows us that, intermediate metabolizers have an increased vulnerability to the development of side effects, i.e. 11/11 (100%) compared to wild types (rapid metabolizers) (0%). On the other hand, the OR = 0.74, a P-value = 0.69; there is a non-significant association between the appearance of side effects due to Cotrimoxazole and the CYP2C9*3 gene in our study (Table V).

In our research work, we studied the polymorphism of the NAT2 and CYP2C9 genes with a view to establishing a procedure manual for the study of NAT2 and CYP2C9 polymorphism in relation to side effects in HIV-positive patients under Cotrimoxazole in Yaoundé. Women were predominantly representative in our study with a participation rate of 67.16%. The mean age ± standard deviation was 43.32 ±28.28, slightly above the result obtained by Carine *et al.,* (2019) in Yaoundé (61% women, and the mean age was 37.5).

Table.1 Primer sequences

Primers	Size	Sequences	References
NAT2F	535 pb	**5'-GCCTCAGGTGCCTTGCATTT-3'**	Kengne *et al.,* 2016
NAT2R		**5'CGTGAGGGTAGAGAGGATAT-3'**	
CYP2C9F	202 pb	**5'-CTGAATTGCTACAACAAATGTGCCA -3'**	ZhiYu *et al*., 2014
CYP2C9R		**5'AGGCTGGTGGGGAGAAGGTCC -3'**	

Table.2 Interpretation of RFLP digestion of NAT2 and CYP2C9 genes

Embarrassed	PCR product size (bp)	Restriction enzyme	Size of the digestion product (bp)	Allele/Genotype/Phenotype
NAT2	535	Kpn I,	483 and 52	NAT2*4
			535	NAT2*5
		Taqα-I	205, 170 and 160	NAT2*4
			330 and 205	NAT2*6
		Bam HI	428 and 107	NAT2*4
			535	NAT2*7
CYP2C9	202	BslNI	105; 75 and 22	CYP2C9*2/3
			127; 105; 75 and 22	CYP2C9*3/3

Table.3 Sociodemographic characteristics

		Minimum	Maximum	Moyenne ± Ecart type
Age		**20**	**60**	**43,32±28,28**
Sex	**Female**	**67,16% (45/66)**		**± 14,84**
	MAle	**31,64%(21/66)**		**± 31,81**
Effects due to Cotrimoxazole	**OUI**	**16,41% (11/66)**		**± 38,89**
	NON	**82,08% (55/66)**		**±7,77**

Table.4 Correlations between NAT2 and the side effects of Cotrimoxazole

		Effects of Cotrimoxazole		OR	IC à 95%	P-value
		NON	YES			
NAT2 phenotypes	FAST	8/55 (14,54%)	3/11 (27,27%)			
	slow	47/55 (85,45%)	8/11 (72,72%)	0,454	0,099- 2,084	0,263
Total		55/66 (83,3%)	11/66 (16,7%)			

Table.5 Correlations between CYP2C9 and side effects of Cotrimoxazole

		COTRI EFFECT		OR	IC 95%	P-value
		NON	YES			
PHENOTYPE CYP2C9*3	Intermediaries	53/55	11/11	0,828	0,741 -0,926	0,692
		(96,4%)	(100,0%)			
	Wild (fast)	2/55	0			
		(3,6%)	(0%)			
Total		55/66	11/66			
		83,33%	16,16%			

Figure.1 Frequency of side effects of Cotrimoxazole

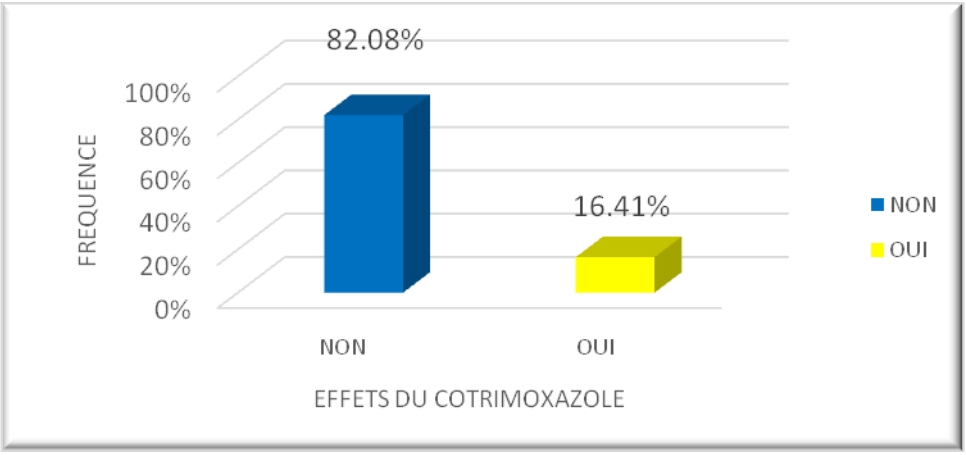
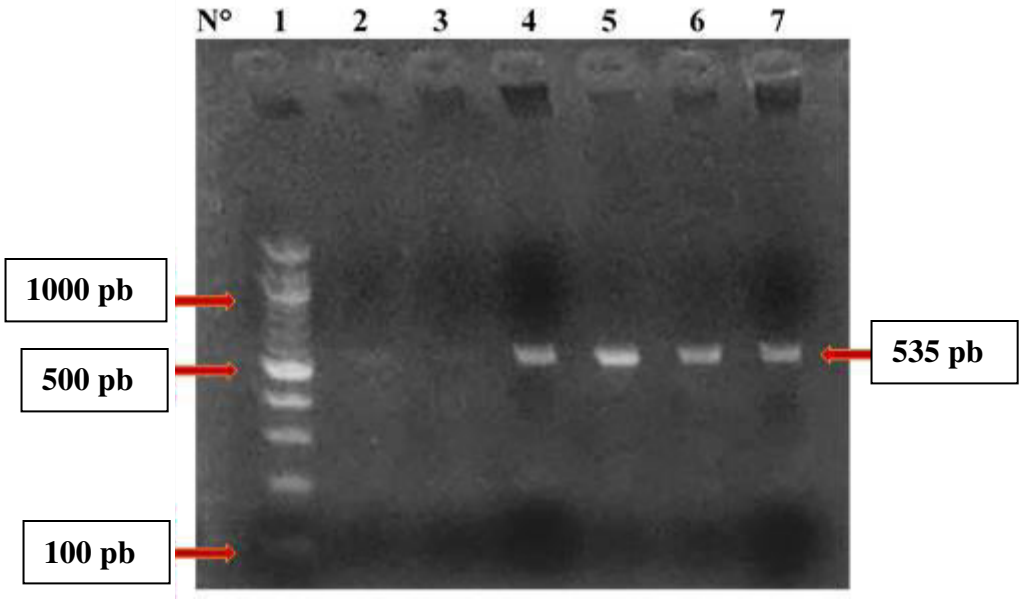


Figure.2 Electropherogram of NAT2 gene amplicons



Legend: No. 1: Molecular weight marker; No. 2, 3, 4, 5, 6, 7: Patients.

Figure.3 Electropherogram pictogram of gene amplicons

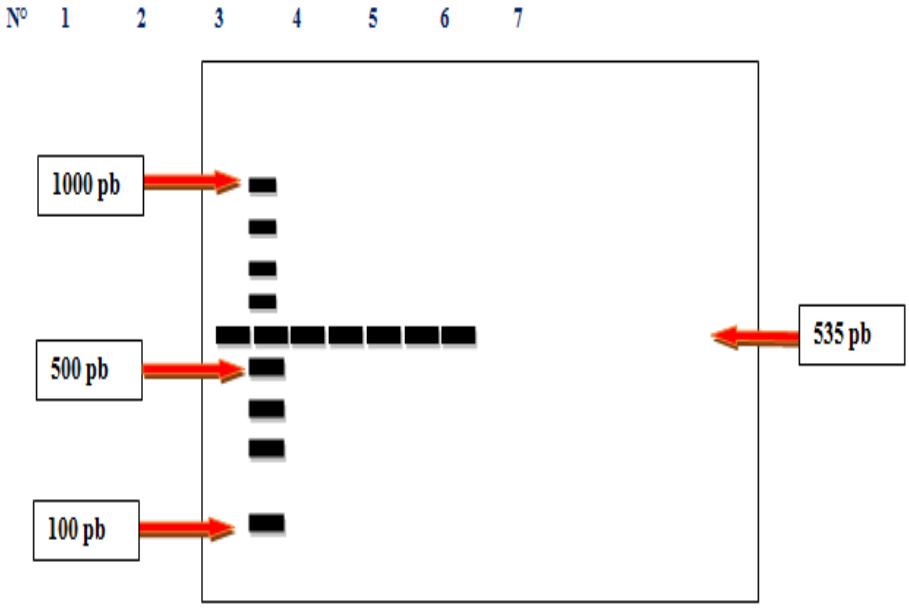
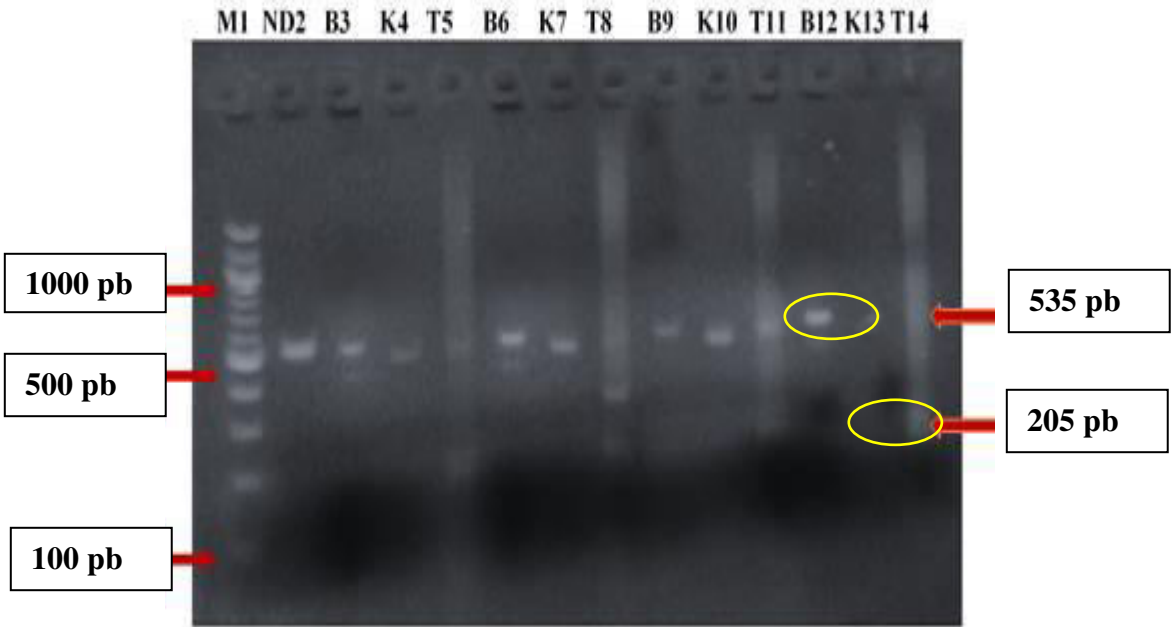


Figure.4 Electrophoresis profile of NAT2 gene digestion with enzymes



Legend: M1 = Molecular weight marker; ND2 = Undigested control sample showing a band at 535 bp

Figure.5 Pictogram of the electrophoresis profile of NAT2 gene digestion

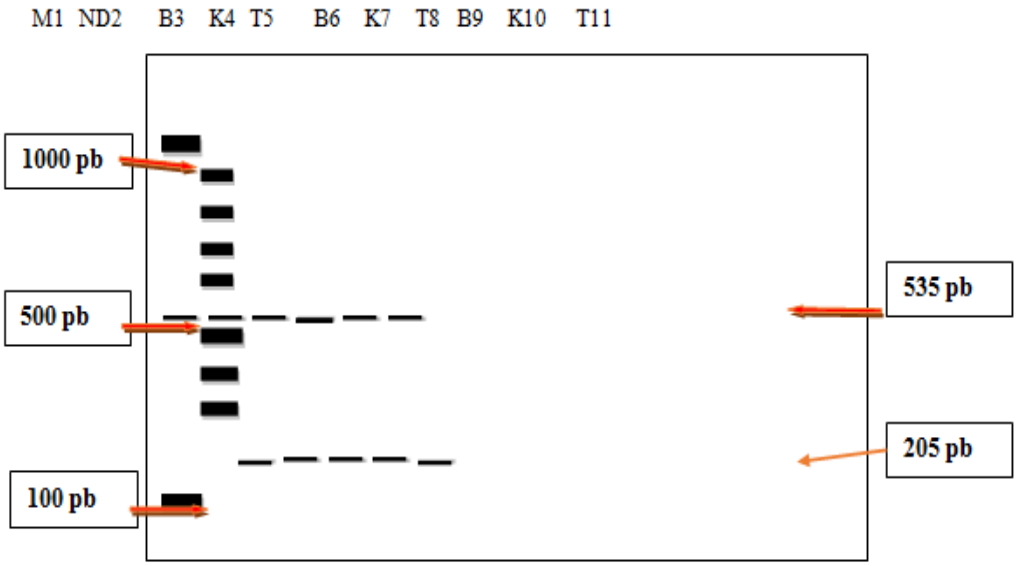


Figure.6 Allelic distribution of the NAT2 gene

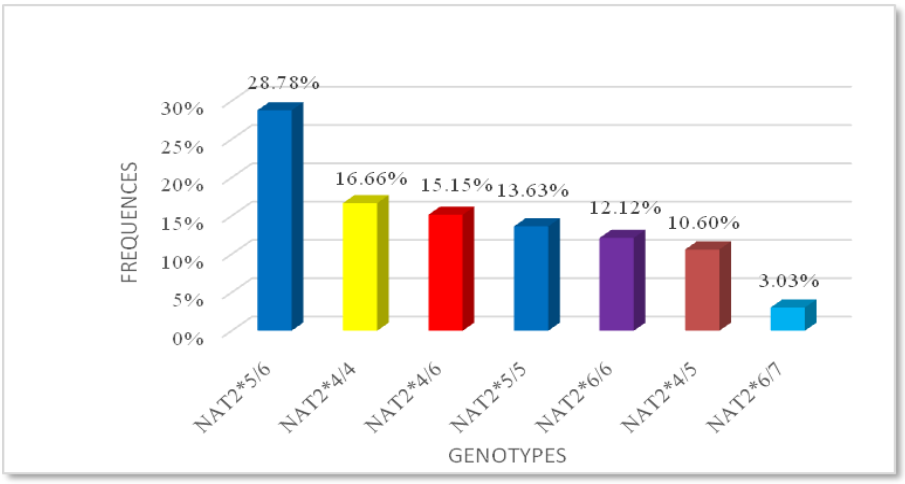


Figure.7 Phenotypic distribution of the NAT2 gene

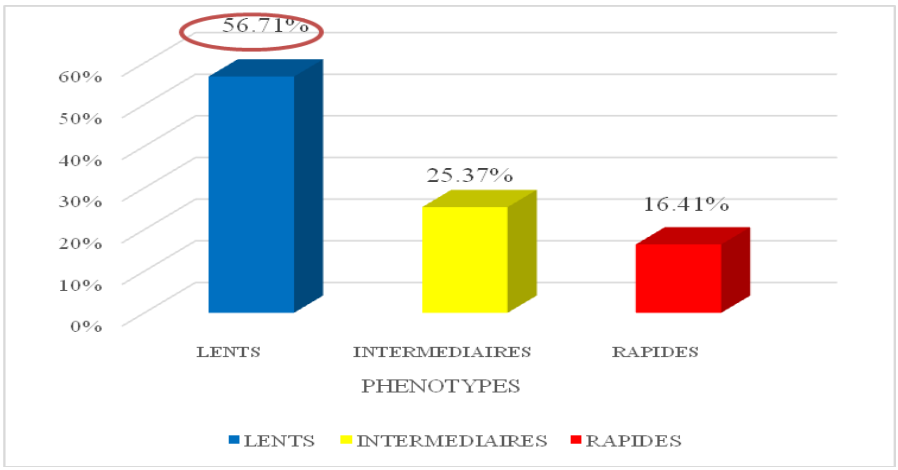


Figure.8 Electrophoresis profile of CYP2C9 gene amplicons.

Figure.9 Electrophoresis pictogram of CYP2C9 gene amplicons

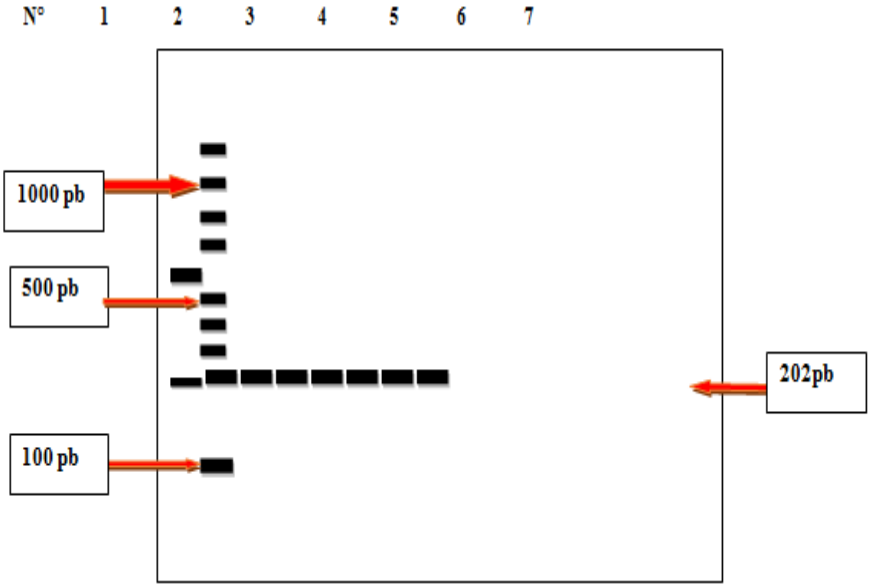
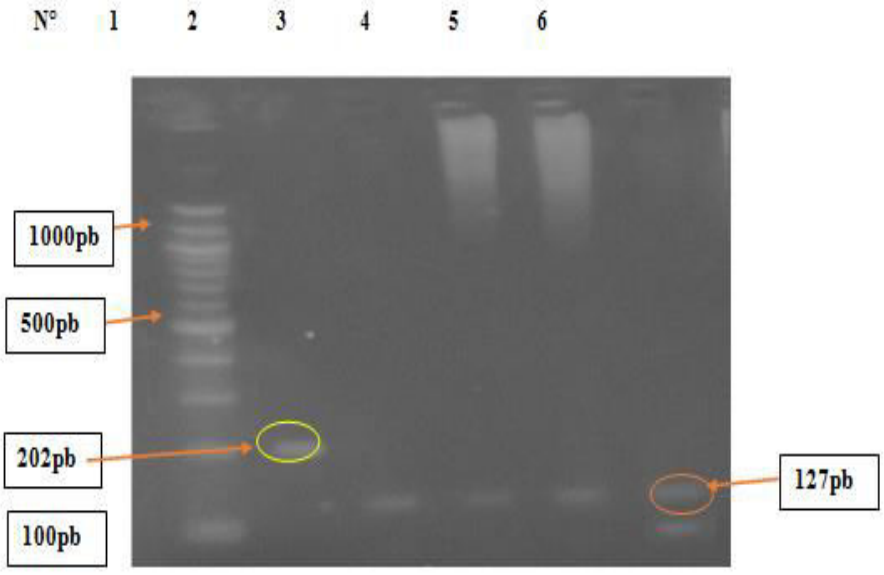


Figure.10 Electrophoresis profile of CYP2C9 gene digestion



Legend: No. 1 = Molecular weight marker; No. 2 = Undigested control sample showing a 202 bp band; No. 3, 4, 5, 6 show bands at 127 (CYP2C9*3); #2, 7 show bands at, 105, (CYP2C9*2)

Figure.11 Electrophoresis pictogram of CYP2C9 gene digestion

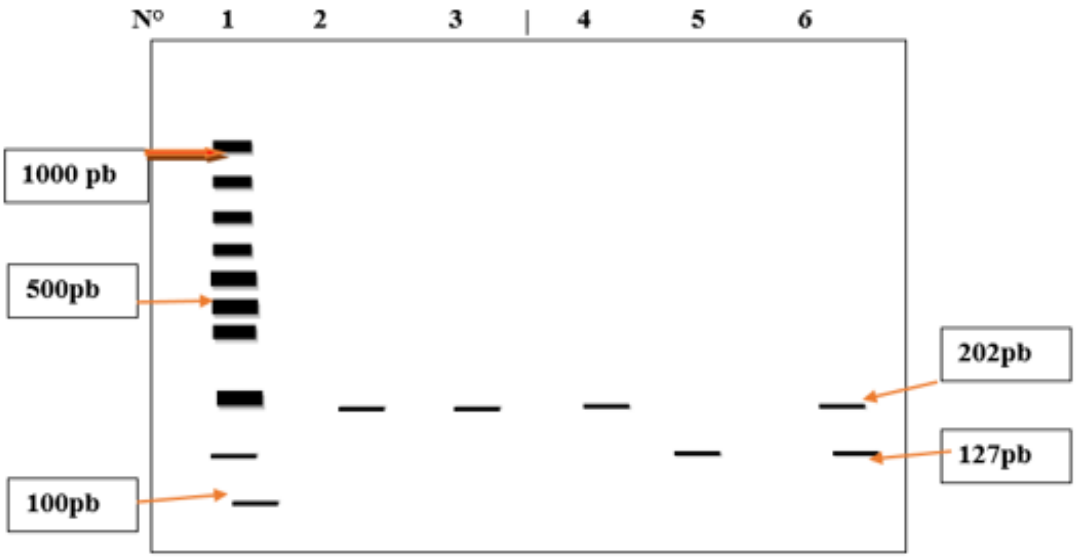


Figure.12 Phenotypic/genotypic frequency of the CYP2C9*3 gene

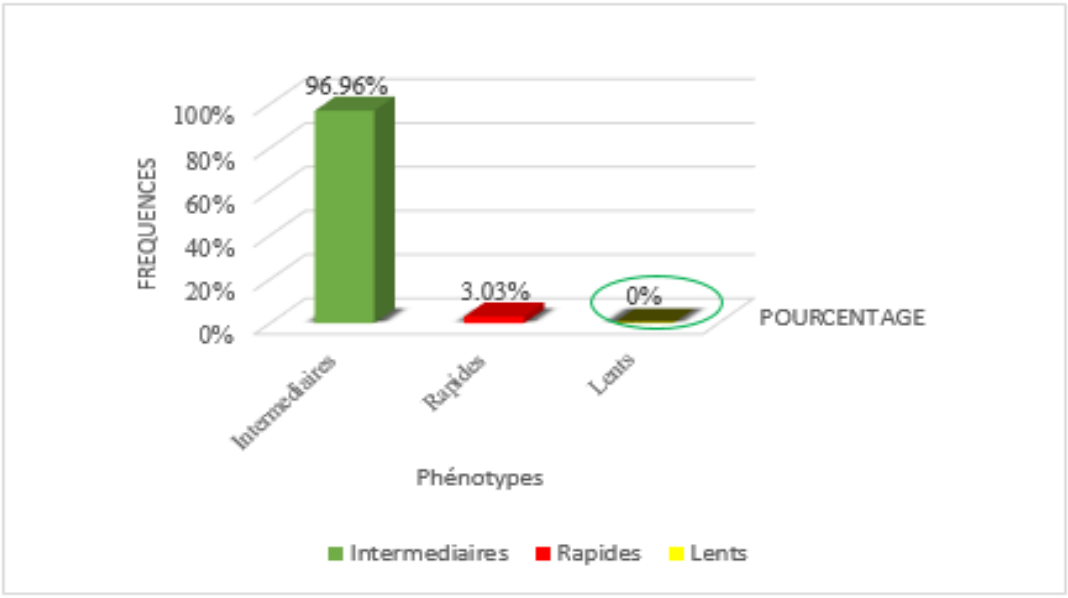
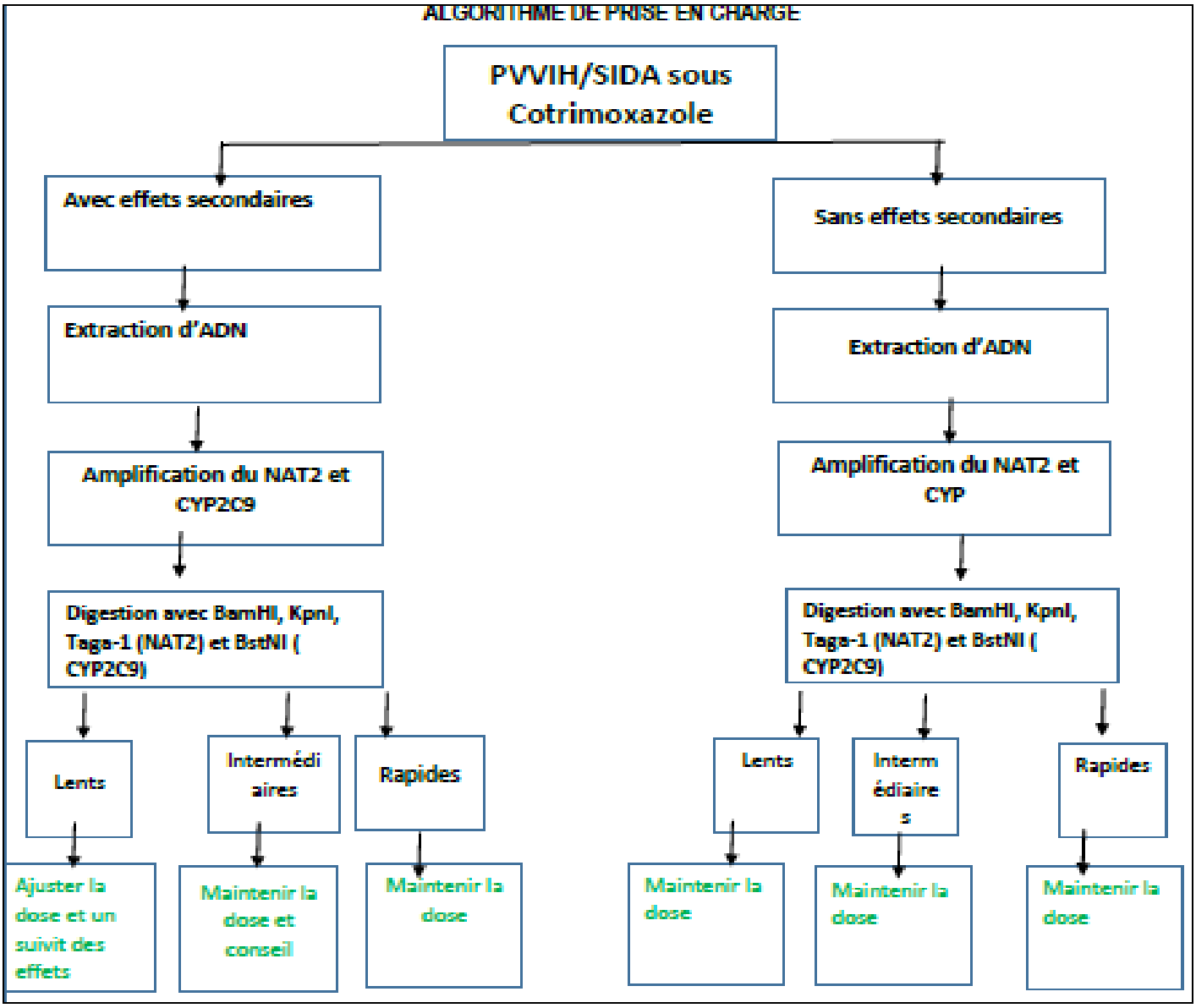


Figure.13



In the present study observed that 7 genotypes with the predominance of NAT2 * 5 / 6, i.e. a frequency of 28.78% (19/66) is similar with the work of Nicolas Ferder *et al.,* (2010) carried out in the North of Cameroon in Garoua, therefore the NAT2 * 5 / 6 genotype predominated at 28.12%. Furthermore, this result does not corroborate the work of Kengne *et al.,* (2016) who obtained in the North and South-West of Cameroon, 9 genotypes with the predominance of NAT2 * 4 / 6 (19%). The high frequency of the slow acetylator phenotype, i.e. 56.71% obtained in our study, is similar to those observed by Kengne *et al.,* (2016) in Cameroon (54%) and Lin *et al.,* (1994) in Singapore subjects at (58%). Unlike, Nicolas Ferder *et al.,* (2010), obtained in their work, slow acetylator phenotypes at 61.4% slightly higher than ours. The phenotypic frequency of slow acetylators obtained in our study is much lower than those of Danadara *et al.,* (2003) in Moroccans (more than 90%) and 73% of the Hong Kong populations O'Neil *et al.,* (2002) and slightly below that of Khan *et al.,* (2013) (50%) in Algeria. Knowledge of the metabolic status of patients is very essential in understanding the differences between populations with regard to their response to treatment (Khan & Chand, 2015). Thus, from our study conducted in Yaoundé, it would be possible for the clinician to consider treatments according to metabolic

status to avoid the occurrence of adverse effects. Indeed, some studies have shown that the slow acetylator phenotype constitutes an independent risk factor for the occurrence of adverse effects related to treatment with Cotrimoxazole compared to the fast acetylator phenotype.

Although this does not exactly answer our study, but our study shows us a non-significant relationship in the occurrence of adverse effects higher in slow acetylators than in fast acetylators, or 72.72% (8/11) versus 27.27% 3/11), with an OR=0.454, P-value=0.263, therefore statistically insignificant. It was observed in another study that there were genetic polymorphisms for allelic variants in the NAT2 and CYP2C9 genes in HIV-positive patients that induce adverse effects (Pirmohamed *et al.,* 2001). This does not corroborate our study conducted in Yaoundé which, out of 66 patients recruited under Cotrimoxazole, the frequency of those having had side effects was 16.7% (11/66) versus 83.3% (55/66), statistically insignificant.

The frequency of CYP2C9 *3 genotype (98.23%) obtained in our study, is largely above the study conducted in Ghana by Wiliam *et al.,* (2009). The correlation result showing a non-significant association between the intermediate phenotype of CYP2C9 and the occurrence of side effects due to Cotrimoxazole. The OR = 0.74, and P-value = 0.38 shows us that there is a non-significant association, but intermediate metabolizers have an increased vulnerability to the side effects of Cotrimoxazole. However, the results of other tests that show no association are not exhaustive due to the small size of our study population. It would be necessary to pay special attention to slow acetylators and intermediate metabolizers patients with regard to their management in the fight against HIV / AIDS with Cotrimoxazole as prophylaxis. In line with our study, the WHO recommends that: taking Cotrimoxazole should be included from the start of HIV infection, until complete recovery of immunity, i.e. a viral load greater than 350 copies/cells.

At the end of this research, the general objective of which was to develop a procedure manual for the study of NAT2 and CYP2C9 polymorphisms on side effects in HIV-positive patients under Cotrimoxazole in Yaoundé.

The frequency of NAT2 and CYP2C9 gene mutation in HIV-positive patients by PCR-RFLP including NAT2 (NAT2*4, NAT2*5, NAT2*6) and CYP2C9 (CYP2C9*2 and CYP2C9*3); 3 alleles in our study with the predominance of the NAT2 *5 allele, i.e. a frequency of 42.42%. 7 genotypes with the predominance of the NAT2*5/6 heterozygote, i.e. a frequency of 28.78% (18/66). Slow acetylators were in the majority, i.e. 56.71%. In our study, we obtained intermediate metabolizers and wild-types of the CYP2C9*2 and CYP2C9*3 genes with a predominance of intermediates, i.e. a frequency of 95.52% (64/66).

Association tests showed us non-significant associations between NAT2/CYP2C9 polymorphisms and the occurrence of side effects related to Cotrimoxazole. However, we must pay particular attention to slow acetylators, given the size of our sample as well as to intermediate metabolizers. Cotrimoxazole prophylaxis could help improve the management of HIV/AIDS patients in Cameroon regardless of metabolic status as recommended by WHO but with particular attention to slow acetylators.

And finally, the present study developed a procedure manual comprising 12 SOPs including: 03 SOPs for administrative research authorizations, 01 SOP for the recruitment of participants, 01 SOP for the preparation of working solutions, 02 SOPs for the collection and transport of samples and 05 SOPs for molecular analysis.

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Author Contributions

Moudine Ouadjonre Francois: Conceived the original idea and designed the model and wrote the manuscript.; Randolph Ngwafor Anye: Conceived the original ideaand Funding Acquisition.; Jean Paul Chedjou: Designed the model and the computational framework and analysed the data.; Calvino Fomboh Tah: Formal Analysis; Ngoum Lesly Ngoum: Methodology; Adawaye Chatte: Writing - Review & Editing, Visualization; Brahim Boy Otchom: Supervision, Review & Editing; Wilfred Fom Mbacham: Supervision, Project Administration, and Funding Acquisition.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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