One-, Two-, and Three-Class Resistance among HIV-Infected Patients on Antiretroviral Therapy in Private Care Clinics: Mumbai, India

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Abstract

HIV-infected patients receiving antiretroviral (ARV) therapy (ART) in India are not all adequately virally suppressed. We analyzed ARV drug resistance in adults receiving ART in three private clinics in Mumbai, India. HIV viral load was measured in 200 patients with the Roche AMPLICOR HIV-1 Monitor Test, v1.5. HIV genotyping was performed with the ViroSeq HIV-1 Genotyping System for 61 participants who had HIV-1 RNA >1000 copies/ml. Genotyping results were obtained for 51 samples. The participants with resistance results were on ART for a median of 24 months and were on their current regimen for a median of 12 months (median CD4 cell count: 217 cells/mm³; median HIV viral load: 28,200 copies/ml). ARV regimens included nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimens ($n = 27$), dual nucleoside reverse transcriptase inhibitors (NRTIs, $n = 19$), protease inhibitor (PI)-based regimens ($n = 3$), and other regimens ($n = 2$). Twenty-six participants (51.0%) were on their first ARV regimen and 24 (47%) reported >95% adherence. Forty-nine participants (96.1%) had resistance to at least one ARV drug; 47 (92.2%) had NRTI resistance, 32 (62.7%) had NNRTI resistance, and four (7.8%) had PI resistance. Thirty (58.8%) had two-class resistance and three (5.9%) had three-class resistance. Four (8%) had three or more resistance mutations associated with efavirenz resistance and two (4%) had two mutations associated with reduced darunavir susceptibility. Almost all patients with HIV-1 RNA >1000 copies/ml had NRTI resistance and nearly two-thirds had NNRTI resistance; PI resistance was uncommon. Nearly 60% and 6% had two- and three-class resistance, respectively. This emphasizes the need for greater viral load and resistance monitoring, use of optimal ART combinations, and increased availability of second- and third-line agents for patients with ARV resistance.

Introduction

ANTIRETROVIRAL (ARV) THERAPY (ART) has dramatically reduced HIV morbidity and mortality and has resulted in substantial recovery of impaired immunologic function among HIV-infected persons all over world.^{1,2} Use of ART is increasing in India, where approximately 2.5 million persons are estimated to be HIV infected.³ Several pharmaceutical companies in India are major global manufacturers of generic ART, and international, national, and local efforts are underway to increase the affordability, access, and use of ART in India.⁴ While government programs are major providers of

free ART, many patients continue to receive HIV care and ART in India's vast and unregulated private sector, which accounts for 81% of India's health care expenditures and provides health care to up to 70% of the Indian population.^{5,6}

The introduction and use of ART in the private sector in India has been understudied. Private sector care may be preferred by some patients as there may be less waiting time, more confidentiality, better rapport with providers, and better individualized care compared to public sector care.^{5,7} However, concerns have been raised about provision of suboptimal ART regimens, lack of adequate therapeutic monitoring, and emergence of ARV drug resistance, which poses a major

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obstacle to the long-term efficacy of ART.^{7–11} Our objective was to determine the prevalence of ARV drug resistance among patients receiving ART in private, urban, outpatient clinics in Mumbai, India. Such data are needed to guide public health policies for HIV treatment in India.

Materials and Methods

Study design

This study was a cross-sectional survey.

Setting and study participants

We analyzed ARV resistance in a cohort that was previously characterized in terms of ART adherence and correlates of virologic suppression.¹² This cohort included HIV-infected individuals 18 years of age or older who were attending one of three private HIV outpatient clinics in Mumbai, India during a 4-month period (January 2005–April 2005). Patients with an acute illness, patients not currently on ART, patients whose ART had been started or changed within the past 3 months, and patients who were unable to provide informed consent were excluded. The study was approved by Johns Hopkins University Institutional Review Board (IRB) and by the local Ethics Committee in Mumbai, India.

Data collection

Attending physicians completed a one-time medical history questionnaire that included data on World Health Organization (WHO) clinical stage, history of opportunistic infections, history of prior ART and duration of each ART regimen, if known, and current ART regimen.¹² After providing informed consent, study participants completed a structured questionnaire via a face-to-face interview with a trained interviewer. The questionnaire included data on demographics, medical history, substance use, and ART adherence using a modified, validated measure (ACTG Baseline Adherence Questionnaire).¹³ Interviews were conducted in English, Marathi, or Hindi.

Laboratory assessments

Ten milliliters of whole blood was processed for complete blood count, quantitative CD4 cell count, and HIV viral load. CD4 cell counts were performed at Metropolis Laboratories in Mumbai, a CAP-certified laboratory. HIV viral load testing was completed for 200 participants and was performed at YRG Care using the Roche AMPLICOR HIV-1 Monitor Test version 1.5. The YRG Care Laboratory was certified by the National Institutes of Health (NIH) Division of Allergy and Infectious Diseases (DAIDS)-sponsored Virology Quality Assurance (VQA) Program. HIV genotyping was performed using the ViroSeq HIV-1 Genotyping System, v2.6 (Celera Diagnostics, Alameda, CA), using plasma samples from 61 participants whose HIV viral load was >1000 copies/ml. HIV genotyping was performed at the National AIDS Research Institute (NARI), which is also VQA certified. Participants were considered to have evidence of ARV resistance if they had a score of “Resistance Possible” or “Resistance Likely” in the ViroSeq report. Because ViroSeq does not provide interpretation for efavirenz or darunavir resistance specifically, we performed a manual review of the

mutations detected and used the Stanford Drug resistance database in combination with the IAS-USA guidelines for interpretation.^{14,15} A random subset of 15 samples was tested in duplicate at the HIV genotyping laboratory at JHU, which is VQA- and CLIA certified; all sequencing data were reviewed at JHU for quality control. HIV genotyping was successful for 51 (83.6%) of the 61 samples. HIV subtyping was performed by phylogenetic analysis of HIV *pol* region sequences obtained with the ViroSeq system. These *pol* region sequences were analyzed using the BioAfrica-REGA HIV Subtyping Tool version 2.0 software (<http://www.bioafrica.net/subtypetool/html/>); all but one participant with resistance test results had subtype C HIV infection; one had subtype B.

Antiretroviral drug abbreviations

The following abbreviations are used in this report; nucleoside reverse transcriptase inhibitors (NRTIs): abacavir (ABC), didanosine (ddI), emtricitabine (FTC), lamivudine (3TC), stavudine (D4T), tenofovir (TDF), zidovudine (AZT); nonnucleoside reverse transcriptase inhibitors (NNRTIs): delavirdine (DLV), efavirenz (EFV), nevirapine (NVP), efavirenz (ETR); protease inhibitors (PIs): amprenavir (APV), atazanavir (ATV), fosamprenavir (FOS), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV), darunavir (DRV), tipranavir (TPV); and fusion inhibitor (FI): enfurvitide (T20).

Statistical methods

Data analyses were performed using STATA Release 10 IC (STATA Corporation, College Station, TX). Analyses included data from the 51 participants with HIV genotyping results. Descriptive statistics were used.

GenBank accession numbers

HIV *pol* region sequences were submitted to GenBank (accession numbers pending).

Results

Study cohort

Of 200 participants who underwent HIV viral load testing, 61 (31%) had a viral load >1000 copies/ml; genotyping was successful for 51 of the 61 samples tested. Compared to persons with VL <1000 copies/ml, persons with VL >1000 copies/ml were less adherent to ART (defined as taking less than 95% of doses in last 4 days; 48% vs. 23%, $p < 0.0001$), were less likely to be on a WHO-approved combination of ART (58% vs. 84%, $p < 0.0001$), and were more likely to have a low CD4 cell count (43% vs. 9%, $p < 0.0001$) and be male (79% vs. 67%, $p = 0.07$). For the group of 51 participants with ARV resistance test results, 41 (80.4%) were male; the median age was 40 years [interquartile range (IQR): 35–46 years] and 42 (82.4%) were WHO clinical stage 3 or 4 (Table 1). The median duration of lifetime ART was 24 months (IQR: 15–36 months), the median duration of the current ART regimen was 12 months (IQR: 6–24 months), the median CD4 cell count was 217 cells/mm³ (IQR: 102–352), and the median viral load was 28,200 copies/ml (IQR: 5220–98,200). The treatment history (number of prior regimens) for these 51 participants, their

TABLE 1. CHARACTERISTICS OF STUDY PARTICIPANTS WITH RESISTANCE TEST RESULTS

Characteristic	Number (%) of participants (n = 51)
Gender	
Male	41 (80.4)
Female	10 (19.6)
WHO clinical stage	
Stage 1/2	9 (17.6)
Stage 3/4	42 (82.4)
Number of prior ART regimens	
0	26 (51.0)
1	11 (21.6)
≥2	14 (27.5)
Current regimen ^a	
2 NRTIs + 1 NNRTI	27 (52.9)
2 NRTIs	19 (37.3)
2NRTIs + 2PIs	3 (5.9)
1 NRTI	1 (2.0)
2 NRTIs + 1 FI ^b	1 (2.0)
Adherence ^c	
<70%	12 (23.5)
≥70–94%	15 (29.4)
≥95%	24 (47.1)

^aNRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; FI, fusion inhibitor.

^bThis subject was highly ART experienced and was on an imported FI (enfuvirtide, T-20).

^cAdherence was assessed using the validated ACTG questionnaire, and was defined as having reported taking 95% or greater of the prescribed doses over the past 4 days.¹³

current regimen type (e.g., two NRTIs + one NNRTI), and their level of self-reported adherence are shown in Table 1.

HIV genotyping results

Forty-nine (96.1%) of 51 participants had at least one ARV drug resistance mutation (Table 2). The two participants who

did not have detectable resistance were both male. One, a 42 year-old male, was receiving his first ARV regimen (a dual NRTI only regimen) with a self-reported adherence of 100%, a CD4 cell count of 82 cells/mm³, and an HIV viral load of 123,000 copies/ml. The other, a 27 year-old male, was receiving his second ARV regimen (a dual NRTI + NNRTI regimen) with a self-reported adherence of <70%, a CD4 cell count of 274 cells/mm³, and an HIV viral load of 3760 copies/ml.

The number of participants with genotypic evidence of resistance to each ARV drug class is shown in Table 2. Eighteen (35.3%) of 51 participants had evidence of resistance to drugs in a single ARV class, including 16 with resistance to ≥1 NRTI, and two with resistance to ≥1 NNRTI. Thirty (58.8%) participants had evidence of resistance to two classes of ARV drugs and three (5.9%) had evidence of resistance to three ARV drug classes (NRTIs, NNRTIs, and PIs). The three participants with three-class drug resistance were all highly ART experienced, with five prior ART regimens. These participants included (1) a 39-year-old male currently on AZT, 3TC, and EFV with a CD4 cell count of 160 cells/mm³ and a viral load of 197,000 copies/ml, (2) a 4-year-old male currently on ddI, 3TC, RTV, and SQV with a CD4 cell count of 199 cells/mm³ and a viral load of 93,900 copies/ml, and (3) a 32-year-old male currently on ddI, 3TC, and T20 with a CD4 cell count of 174 cells/mm³ and a viral load of 229,000 copies/ml. The number of participants with evidence of resistance to individual ARV drugs is shown in Table 3. Four patients had evidence of at least one major PI mutation. Two were currently on a PI-based regimen (one was on SQV/RTV/3TC/ddI but had a prior history of being on SQV/NFV/ddI/3TC and then IDV/RTV/d4T/3TC; the other was on AZT/3TC/SQV/RTV since 2004). The two other with major PI mutations were currently on non-PI-based regimens (one was on T20/3TC/ddI but had a prior history of SQV/d4T/3TC, IDV/RTV/AZT/3TC, LPV/RTV/d4T/ddI, and LPV/RTV/AZT/ddI regimens and the other was on AZT/3TC/EFV but had a history of IDV/RTV/d4T/3TC and LPV/RTV/d4T/3TC regimens).

The most common NRTI resistance mutations detected were M184V (90.2%), D67N (35.3%), M41L (25.5%), and

TABLE 2. PROPORTION OF PARTICIPANTS WITH LIKELY OR POSSIBLE ARV DRUG RESISTANCE BY ADHERENCE LEVEL AND BY REGIMEN TYPE^a

Type of drug resistance	All subjects (n = 51)	Adherence level			Type of ART regimen			
		Adherence ≤95% (n = 27)	Adherence >95% (n = 24)	First ART regimen (n = 26)	2 NRTIs + 1 NRTI (n = 27)	2 NRTIs (n = 19)	2 NRTIs + 2 PIs (n = 3)	Other (n = 2)
≥1 ARV drug	49 (96.1)	26 (96.2)	23 (95.8)	25 (96.1)	26 (96.3)	18 (94.7)	3 (100.0)	2 (100)
≥1 NRTI	47 (92.2)	25 (92.6)	22 (91.7)	24 (92.3)	24 (88.9)	18 (94.7)	3 (100.0)	2 (100)
≥1 NNRTI	32 (62.7)	14 (51.9)	18 (75.0)	11 (42.3)	26 (96.3)	4 (21.1)	1 (33.3)	1 (50.0)
≥1 PI	4 (7.8)	2 (7.4)	2 (8.3)	0 (0.0)	1 (3.7)	0 (0.0)	2 (66.7)	1 (50.0)
Two-class	30 (58.8)	15 (55.6)	15 (62.5)	10 (38.5)	23 (85.2)	4 (21.1)	1 (33.3)	1 (50.0)
Three-class	3 (5.9)	1 (3.7)	2 (8.3)	0 (0.0)	1 (3.7)	0 (0.0)	1 (33.3)	1 (50.0)

^aThe number and percentage (in parentheses) of participants are shown who had resistance to any antiretroviral (ARV) drug (≥1 ARV drug), to one or more nucleoside reverse transcriptase inhibitor (≥1 NRTI), to one or more nonnucleoside reverse transcriptase inhibitor (≥1 NNRTI), and to one or more protease inhibitor (≥1 PI), as well as those with resistance to two or three classes of ARV drugs (two-class, three-class). The same information is provided for participants by their self-reported adherence level, receiving their first ART regimen, and for the subset of participants receiving different types of ART regimens. Patients with ≤95% adherence were more likely to be on their first ART regimen [17 (63.0%) of 27 vs. 9 (37.5%) of 24, $p < 0.01$] and less likely to be on their third or more regimen [5 (18.5%) of 27 vs. 9 (37.5%), $p < 0.01$].

TABLE 3. DETECTION OF LIKELY OR POSSIBLE ARV DRUG RESISTANCE AMONG 51 STUDY PARTICIPANTS^a

ARV drug	Evidence of resistance, n (%)
NRTI	
ABC	20 (39.2)
ddI	18 (35.3)
FTC	47 (92.2)
3TC	47 (92.2)
d4T	21 (41.2)
TDF	13 (25.5)
AZT	22 (43.1)
NNRTI	
DLV	32 (62.7)
EFV	31 (60.8)
NVP	32 (62.7)
PI	
NFV	4 (7.8)
IDV	4 (7.8)
LPV	3 (5.9)
ATV	3 (5.9)
SQV	4 (7.8)
RTV	4 (7.8)
FOS	3 (5.9)
TPV	3 (5.9)
APV	3 (5.9)

^aThe number and percentage (in parentheses) of participants whose testing revealed likely or possible ARV resistance to each antiretroviral (ARV) drug, according to the ViroSeq report; these numbers were combined to indicate the total number and percentage of participants with evidence of ARV drug resistance. ARV drug abbreviations are defined in the Materials and Methods section.

T215F/Y (31.4%); 19 (37.3%) of the participants had three or more thymidine analog mutations (TAMs) (Table 4). The most common NNRTI resistance mutations detected were K103N/S (27.5%), G190A/S (21.6%), K101E/P (21.6%), and Y181C/I (19.6%). Version 2.6 of the ViroSeq HIV Genotyping System does not provide an interpretation for the NNRTI, etravirine (ETR), or the PI, darunavir (DRV). However, manual review of the mutations detected revealed that four (8%) participants had three or more mutations associated with ETR resistance and two (4%) had two mutations associated with reduced DRV susceptibility. All of the samples had polymorphisms in HIV protease that are commonly seen in non-subtype B HIV. Some participants also had major PI resistance mutations, as defined by the Stanford HIV Drug Resistance Database.¹⁴ The most common major PI resistance mutations detected were I54V (5.9%), V82A/F (3.9%), and L90M (3.9%).

Discussion

In India, nearly 200,000 persons are now receiving ART¹⁶ [B.B. Rewari, National AIDS Control Organization (NACO), personal communication]. However, relatively little is known about the prevalence of ARV drug resistance in India, particularly among those being treated in the private sector where an individualized approach is taken and where both standard and nonstandard ARV regimens are readily available. Limited evidence suggests that some patients who pay for ART and medical care out-of-pocket may have difficulty

TABLE 4. DETECTION OF SPECIFIC ARV DRUG RESISTANCE MUTATIONS AMONG 51 STUDY PARTICIPANTS^a

Mutation	Number of participants with mutations (%)
NRTI	
M184V	46 (90.2)
TAMs	
M41L	13 (25.5)
D67N	18 (35.3)
K70R	10 (19.6)
L210W	2 (3.9)
T215F/Y	16 (31.4)
K219Q/E	6 (11.8)
M41L + L210W + T215F/Y	2 (3.9)
D67N + K70R + K219Q/E	6 (11.8)
≥3 TAMs	19 (37.3)
Non-TAMs	
K65R	1 (2.0)
K70E	1 (2.0)
L74V/I	5 (9.8)
V75T/M	3 (5.9)
Multi-NRTI	
V75I	2 (3.9)
NNRTI	
A98G	7 (13.7)
K101E/P	11 (21.6)
K103N/S	14 (27.5)
V106M	4 (7.8)
V108I	6 (11.8)
Y181C/I	10 (19.6)
Y188L	4 (7.8)
G190A/S/E	11 (21.6)
F227L	1 (2.0)
M230L	1 (2.0)
Major PI mutations	
M46I	1 (2.0)
G48V	1 (2.0)
I54V	3 (5.9)
G73S	1 (2.0)
V82A/F	2 (3.9)
I84V	1 (2.0)
L90M	2 (3.9)

^aThe table shows the number of study participants with specific ARV drug resistance mutations. Abbreviations for drug classes are provided in the Materials and Methods section. NRTI mutations are classified as thymidine analog mutations (TAMs), non-TAMs, and multi-NRTI mutations (mutations conferring resistance to multiple NRTIs). Other NRTI resistance-associated mutations were detected, including accessory mutations (E44A/D, *n* = 5; V118I, *n* = 5) and mutations selected in treatment (T69A/D, *n* = 4; K219N, *n* = 1). Four (8%) had three or more resistance mutations that have been associated with significantly reduced susceptibility to etravirine, a second-generation NNRTI (data not shown). Major PI mutations have been shown to reduce PI susceptibility *in vitro* and/or to reduce the virologic response to therapy; some of these mutations are relative contraindications to the use of specific PIs.¹⁴ Other PI resistance-associated mutations were detected, including accessory mutations (L10I, *n* = 1; I13V, *n* = 3; K20R, *n* = 5; M36I, *n* = 43; K43T, *n* = 1; K65R, *n* = 4; A71V/T, *n* = 4; V75I, *n* = 2; V77I, *n* = 1), and F53L, *n* = 1.

adhering to their treatment regimens.^{12,17} Furthermore, some patients may be taking and/or their providers may be prescribing inappropriate regimens (e.g., a single NNRTI or dual NRTIs) that are inconsistent with the recommendations of the WHO and the Indian government's NACO.^{8,12,17,18}

Small studies of recent seroconverters and ARV-naïve patients in India have shown that there is no or limited drug resistance; however high levels of NRTI and NNRTI resistance among small numbers of ARV treatment-experienced patients in western and southern India have been observed.^{19–23} Some limitations of those studies are that several different methods were used to assess ARV drug resistance and most did not assess ARV adherence, prevalence of two- or three-class ARV resistance, or resistance by current ARV regimen type.^{21–23} Our survey of 200 patients receiving ART at private clinics revealed that about one-third of patients had viral loads >1000 copies/ml. Among the 51 participants with resistance test results, almost all participants had evidence of NRTI resistance and about two-thirds had evidence of NNRTI resistance. PI resistance was uncommon, most likely because PIs are rarely used in India because of their cost. More than 50% of the participants with drug resistance mutations had two-class resistance and 5.9% had three-class resistance, specifically to NRTIs, NNRTIs, and PIs. One of these patients was on imported T-20 and therefore may have actually had four-class resistance, but we were unable to verify the presence of resistance mutations to fusion inhibitors. Other studies of ARV drug resistance in individuals with non-subtype B HIV infection in resource-limited settings have also observed that many patients have detectable HIV RNA and have high rates of ARV drug resistance. For example, in Mozambique where subtype C and CRF08 recombinant HIV are common, 42 (28%) of 149 patients on first-line ART for a mean of 23 months had detectable viral loads.²⁴ In Tanzania, where subtype A, C, and D HIV are common, 32% of patients on ART for a median of 12 months had viral loads >400 copies/ml.^{24,25} In South Africa, where subtype C predominates, 83.5% of patients failing a first-line ART regimen had at least one major resistance mutation, 64.3% had two-class resistance, and 2.6% had three-class resistance.²⁶ Higher rates of PI resistance were detected in Burkina Faso, where CRF06_cpx and CRF02_AG HIV strains predominate; there, among 75 who were failing ART, 85% had NRTI resistance, 76% had NNRTI resistance, and 40% had PI resistance.²⁷ The higher rate of PI resistance in Burkina Faso reflects frequent use of PIs in that region; approximately 40% of patients in the study cited had a history of PI exposure.

In our study, more than 37% of participants were receiving dual NRTIs only. Such suboptimal regimens rapidly result in clinically relevant NRTI resistance, compromising the long-term success of ART. Studies have shown that some patients have poor understanding of ART and may be unaware of whether they are on ART or not, let alone whether these are appropriate combinations of ARVs. Patients also do not always purchase the full combination of ARVs as prescribed if they are paying out of pocket for these medicines.^{6,8} Furthermore, studies have shown that physicians are more likely to prescribe nonsuppressive ART regimens for patients who they feel may have difficulty paying for ART, who have limited ART experience, or who may have a limited understanding of ART.^{7,9,28–30}

The drug resistance mutations we detected in this Indian cohort are similar to those that have been described in individuals from other countries who have subtype C infection.^{21,26,31} For example, in a South African study, M184V was the most common NRTI mutation and K103N was the most common NNRTI mutation; V106M was detected more often

than V106A.²⁵ We also found that nearly one-third of the participants had three or more TAMs; the most common TAMs detected were M41L (26%), D67N (37%), K70R (20%), and T215F/Y (33%). Interestingly, we identified only one subject with the K65R mutation; that mutation has been identified more frequently in other studies of ARV drug resistance in subtype C infections in Africa.^{26,32} This may reflect the fact that ddI and TDF, which favor development of this mutation, were rarely included in the ART regimens in our cohort or may reflect potential differences between subtype C strains in India and Africa.

In terms of future ARV options, we found 8% had three or more NNRTI mutations associated with markedly reduced efavirine susceptibility^{14,33} and 4% had two of the PI mutations associated with reduced darunavir susceptibility, which suggests minimal or “low-level” resistance to darunavir. All four patients who had evidence of PI resistance were either currently on PI-based regimens or had prior PI experience. A boosted PI such as lopinavir or darunavir therefore would work very well as a component of a second-line or third-line regimen, particularly because they have high barriers to resistance and high antiviral activity (i.e., high inhibitory potential).^{14,34,35}

The WHO and NACO recommend that second-line ART regimens include NRTIs. However, in the private clinic settings assessed and where an individualized approach was used, many participants had evidence of high-level NRTI resistance; resistance data were not used to guide ART regimen selection in this cohort, and many of the participants were maintained on NRTI- and NNRTI-containing regimens with inadequate virologic suppression. Therefore, there is a clear need for rationale use and sequencing of ARVs in the private sector clinics and ongoing national drug resistance surveillance programs that monitor ARV drug resistance in India must include both private and public sector settings. Data obtained by such programs can help guide treatment recommendations and increase awareness and understanding of ARV drug resistance among physicians and patients.

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Disclosure Statement

No competing financial interests exist.

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