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Epidemiological serosurvey and molecular characterisation of sexually transmitted infections among 1,890 sheltered homeless people in Marseille: cross-sectional one day-surveys (2000-2015)

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Abstract

Objectives. We observed the prevalence and distribution of potential risk factors for sexually transmitted infections (STIs) among Marseille homeless population.

Methods. Over the 2000-2015 period, we enrolled 1890 sheltered homeless adults and collected serum samples. Markers of hepatitis B and C viruses (HBV, HCV) and *Treponema pallidum* were searched using the CMIA testing. Positive HBsAg or anti-HCV samples underwent sequencing; positive anti-*T. pallidum* sera were subjected to the RPR test.

Results. The overall prevalence of HBsAg, anti-HBs, anti-HBc, anti-HCV and anti-*T. pallidum* (by CMIA and RPR) was 4.1 %, 22.9%, 35.5%, 5.3% and (6.8%, 1.0%), respectively. We found a significantly higher prevalence of HBsAg and anti-*T. pallidum* among individuals born in sub-Saharan Africa (or Asia) compared to those born in Europe. Being older (>42 years), toxicomania status, cannabis use and underweight status (compared to normal status) were independent factors associated with HCV seropositivity. Using sequencing, we obtained a substantial diversity of HBV and HCV genotypes. One HCV sequence harbouring a L31M substitution in the NS5a protein may be associated with reduced drug sensitivity.

Conclusions. The positive relationship between toxicomania and HCV suggests the need for effective prevention programmes including health education activities and addiction treatment.

Introduction

Homelessness is an increasing social and public health concern in both developing and developed countries. Given their lack of health insurance coverage leading to a limited access to healthcare, homeless people are particularly exposed to infectious diseases which are more severe than in the general population [1]. In addition, homeless people are disproportionately affected by substance and alcohol abuse and the consequences of this on their health negatively impacts their general well-being and mental health [1].

As blood- and sexually-transmitted pathogens, the hepatitis B virus (HBV), hepatitis C virus (HCV), and *Treponema pallidum* can be diffused via blood transfusion, vertical transmission and sexual practice [2]. Homeless people are at high risk of these diseases as the result of injected drug use, unprotected sexual activity and financial and nutritional poverty [3,4]. Globally, epidemiological surveys carried out among homeless populations have shown a prevalence of 2.2%–36.2% for HCV infection [5,6]. The prevalence of past HBV infection (positive anti-HBc antibodies) ranged from 10.4% to 80.3% and that of ongoing infection (positive HBsAg) ranged from 0.4% to 4.7% [6,7]. Syphilis was observed in approximately 0.5% of homeless people in Iran (2012), in 1.1% in England (2004), in 7% in Brazil (2000-2011) [8,9], and in 33.2% of Brazilian female prisoners with a history of homelessness (2014-2015) [10]. Human immunodeficiency virus (HIV) infection is frequent in the homeless population and has been extensively studied [5].

Homelessness affects more than 1,500 persons on any given night in Marseille, including 800 who sleep on the city's streets and approximately 600 who are living temporarily in municipal shelters, which have a high turnover [11]. Medical care of sheltered homeless people, who are a highly mobile population and mostly of migrant origin, often remains fragmented across multiple inpatient and outpatient settings. The screening and treatment of infectious diseases, notably due to sexually-transmitted pathogens (STPs), in the homeless population is thus

necessary. The purpose of the current study was, therefore, to investigate the seroprevalence of several STPs (other than HIV infection) and their risk factors among homeless people living within shelters in Marseille, France, between 2000 and 2015.

Materials and methods

Ethics

The Marseille Institutional Review Board-Ethics Committee approved and reviewed the design of this retrospective study (Protocol: CCPCRB 99-76 [period 2000-2009] and 2010-A01406-33 [after 2010]). Eligible participants were confidentially interviewed and gave their informed consent. Data were anonymised and de-identified prior to analysis.

Patient and sample design

In our cross-sectional one-day surveys, adult homeless people who presented at two shelters (A and B) in Marseille, France, were recruited on a voluntary basis during the winter in 2000, 2001, 2002, 2003, 2005, 2006, 2010, 2011, 2013, and 2015. The inclusion criteria were being 18 or over and being willing to participate. Participants were asked to complete a specially designed questionnaire to provide information on demographics and substance abuse, and they were physically examined by medical doctors. Trained medical personal collected a blood sample for serological testing from each patient. Those who tested positive for these infections were counselled about their infections and referred to primary care.

Serological assays

A 5 ml blood sample was drawn from each subject using a sterile vacutainer tube (Becton Dickinson, Rutherford, NJ, USA) and the serum was separated using centrifugation (at 2900 g for 15 minutes). All sera were screened for the presence of antibodies against HBV, HCV and syphilis. The fully automated chemiluminescent microparticle immunoassay (CMIA, Abbott, Diagnostics Division, Wiesbaden, Germany) was used to detect the following serological

markers: hepatitis B surface antigen (HBsAg), hepatitis B surface antibodies (anti-HBs), anti-hepatitis B core total antibodies (anti-HBc), antibodies to hepatitis C virus (anti-HCV), and antibodies to *T. pallidum* (anti-*T. pallidum*), according to the manufacturer's instructions. The criteria for seropositivity and seronegativity for each marker are detailed in Supplementary Table 1. Samples which were positive for HBsAg underwent additional testing for HBsAg positivity using a neutralisation assay (Abbott, Diagnostics Division) according to the manufacturer's guidance. Depending on the results of different combinations of HBV serological markers, patients were defined as: (i) chronically or acutely infected, (ii) susceptible (absence of immunity), (iii) immune due to natural infection, (iv) immune due to hepatitis B vaccination, or (v) presenting a past resolved infection (Supplementary Table 2). For HCV, patients testing positive for anti-HCV antibodies were considered as having been infected during their lifetime. Subsequently, samples showing seropositivity for HBsAg and anti-HCV antibodies were investigated using molecular assays to test for replication and ongoing infection, respectively. For *T. pallidum*, samples which tested positive using CMIA were further tested using the rapid plasma regain test (RPR) (RPR Latex Test Kit, Diagnostic Automation/Cortez Diagnostics, Inc., USA) according to the manufacturer's instructions.

DNA and RNA extraction

Total DNA and RNA were automatically extracted from 200 μL of serum using the EZ1 Advanced XL (Qiagen, Hilden, Germany) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's recommendations. The obtained extracts were stored at -80°C.

Conventional PCR amplification and DNA sequencing

We characterised the full length HBsAg and reverse transcriptase (HBsAg/RT) genes (for HBV) and large fragments of NS4B and NS5A genes (for HCV). Only samples with seropositive results for HBV and/or HCV were further tested using standard PCR (as described in

Supplementary Data). The purified PCR products were sequenced using specific primers and the BigDye Terminator® version 1.1 cycle sequencing ready reaction mix (Applied Biosystems, Foster City, CA). All primers and in-house protocols used in this study have previously been described (as shown in Supplementary Table 3). The sequencing reactions were purified with SephadexG-50 Superfine on MAHVN 45–50 plates (Millipore, Molsheim, France) and then sequenced on the Applied Biosystems 3130 platform (ABI PRISM, PE Applied Biosystems, USA). For each pathogen, the sequences obtained were edited and assembled using Chromas Pro1.7.7 software (Technelysium Pty Ltd, Australia). HBV (or HCV) genotypes were determined by phylogenetic analysis using a set of reference HBV (or HCV) sequences available from the NCBI GenBank or from our institute's local HBV (or HCV) sequence bank of the population of Marseille and the Max-Planck-Institut für Informatik [12] sequence databases, using the Mega 7.0 software (https://www.megasoftware.net). In these websites, the nucleotide sequences obtained were simultaneously translated into amino acid (aa) sequences then compared with reference HBV (or HCV) sequences of the same genotype in order to determinate aa substitutions previously described in association with reduced antigenicity or susceptibility to anti-HBV (or HCV) drugs. These sequences are available in GenBank (GenBank accession nos. **MK840522** to **MK840537** [for HBV] and **MK816407** to **MK816412** [for HCV]).

Statistical analysis

Statistical procedures were performed using STATA 11.1 software (StataCorp LLC, USA). Statistical differences in baseline characteristics were evaluated by Pearson's chi-square or Fisher's exact tests as categorical variables. A two-tailed p-value <0.05 was considered as statistically significant. Three separate models (HBV, HCV and *T. Pallidum*) were created. A separate multivariate logistic regression analysis was used to identify independent risk factors for sero-prevalence of each pathogen. Only variables with a prevalence ≥5.0% by descriptive

analysis were used to examine associations between multiple factors and sero-prevalence of each pathogen. The results were presented by percentages and odd ratio (OR) with 95% CI estimations. The initial model included variables presenting a p-value <0.2. The stepwise regression procedure and likelihood-ratio tests were applied to determine the final model.

Results

Participant characteristics (Table 1)

The demographic characteristics of the study sample are presented in Table 1. The study included a total of 1,890 individuals who completed questionnaires; of them, 1,718 (90.9%) provided blood samples. There were 1,779 (95.2%) men and 90 (4.8%) women with a mean age (± standard deviation [SD]) of 43.1 (±16.4) years. Homeless people were mainly born in North Africa (n=873, 46.5%), Europe (n=853, 45.5%), and sub-Saharan Africa (n=115, 6.1%). The average duration of homelessness (±SD) was 3.9 (±6.9) years and about 45% had experienced homelessness for more than one year (defined as chronic homelessness). For migrants, their mean duration of residence in France (±SD) was 13 (±16.7) years. Frequent alcohol abuse and tobacco smoking was reported by 38% and 78% of individuals, respectively. Current cannabis use was reported by 115 individuals (of the 610 who were asked, 18.9%). Drug addiction status, was uncommon, being reported by 47 individuals (6.1%). A history of blood transfusion was reported by 102 individuals (of the 756 who were asked, 13.5%); of those, 13 participants (1.7%) had received a transfusion before 1992. About 63.5% of patients showed normal weight, 75 (6.4%) were underweight, 278 (23.8%) were overweight and 74 (6.3%) were obese.

Serological patterns and risk factor analysis: multivariate models (Table 1-3 & Supplementary Figure 1-2)

The overall prevalence of HBsAg, anti-HBs, anti-HBc, anti-HCV, and anti-*T. pallidum* during the study period was 4.1% (54/1310), 22.9% (301/1312), 35.5% (379/1059), 5.3% (90/1601) and 6.8% (106/1564) via CMIA testing, respectively. Of the 1053 samples tested for all three HBV markers, more than half showed an absence of immunity against HBV. Only 69 individuals (6.5%) had acquired immunity through vaccination, whereas 19 (17.9%) had immunity due to natural HBV infection, 189 (17.8%) had a past resolved infection, and 54 (4.1%) had an ongoing (chronic or acute) infection. Of the 106 patients considered as seropositive for anti-*T. pallidum* via CMIA testing, 15 (of 1564, 1%) tested positive via RPR testing. The most frequent dual infection was observed with *T. pallidum* and HCV in seven cases (0.5%).

The sero-prevalence of these pathogens did not significantly vary over time. In multivariate analysis, being born in sub-Saharan Africa (or Asia) continued to be associated with higher HBsAg and anti-*T. pallidum* seropositivity rates when compared to those born in Europe. Being over the age of 42, addicted to drugs, smoking cannabis and being underweight remained associated with anti-HCV seropositivity.

Genotypic patterns of HBV (Figure 1 & Supplementary Table 4)

HBV genotyping was performed on 44 available sera from 44 HBsAg-positive subjects. We succeeded in amplifying the gene from 16 subjects (36%). The phylogenetic tree showed that HBV sequences of our homeless people clustered with genotype A (HBV-A), D (HBV-D) or E (HBV-E) reference sequences. Thus, we detected the HBV-A in 2 cases (12.5%; A2=2), HBV-D in 9 cases (56.2%; D1=3, D2=1, D3=1, D4=2, D7=2), and HBV-E in 5 cases (31.2%). Overall, the mean (±SD, range) nucleotide identity between our 16 HBV sequences characterised and their best match available in the GenBank and in our local sequence database was 98.7% (±0.8%, range 96.8%-99.7%) and 97.1 (±1.0%, range 95.8%-98.7%), respectively. The HBV sequences recovered from homeless people were scattered across the

phylogenetic tree, and were neither clustered between each other (with one exception) nor more frequently clustered with locally-acquired sequences compared to GenBank sequences.

The deduced HBsAg aa sequences showed that aa substitution T127I/P was most frequently encountered, being present in nine sequences (of 16, 56.2%) when compared to reference sequences. Patient SDF097 had 10 substitutions (of 226 HBsAg aa, 4.4%). Only one patient showed a substitution (D144E) known to confer altered antigenicity of the HBsAg protein. However, no substitution was found that is described to decrease susceptibility to HBV antiviral drugs.

Genotypic patterns of HCV (Figure 2 & Supplementary Table 5)

HCV RNA could be amplified from six of the 30 (20%) sera available from 30 anti-HCV-positive subjects. Two of six (33.3%) HCV sequences belonged to subtype 1a (HCV-1a), two (33.3%) to subtype 1b (HCV-1b), one (16.6%) to subtype 3a (HCV-3a), and one (16.6%) to subtype 4d (HCV-4d). Overall, the mean (±SD, range) nucleotide identity between the six HCV sequences characterised in this study and their best match available in the GenBank and in our local sequence database was 96.2% (±1.5%, range: 93.6%-97.8%) or 94.2% (±1.9%, range: 92.3%-96.7%), respectively. The HCV sequences recovered from homeless people were scattered across the phylogenetic tree and were neither clustered close to one another nor more frequently clustered with locally acquired sequences than with GenBank sequences.

As regards specific aa substitutions, the L31M mutation that was previously described to be associated with reduced susceptibility to Daclatasvir, Elbasvir or Ledipasvir drugs was present in one patient (SDF108). This patient received a blood transfusion in 1975 and reported no history of HCV infection at the time of recruitment.

Discussion

Our study documented the prevalence of sexually transmitted infections (STIs) among sheltered homeless adults in Marseille du the period 2000-2015 and their risk factors. The key

strengths of this study are its long duration and large population. We found a high prevalence of past or current HBV infection (39.8%). The overall HBsAg prevalence was 4.1% in the homeless population studied, which is higher than the 1.1% prevalence in the general local population over the same period [13]. We found that originating from Asia or sub-Saharan Africa was an independent risk factor for HBsAg positivity. However, positivity rates were still high in those originating from North Africa (3.2%) and Europe (4.0%). Such a high rate of active infection may be due to the low proportion of homeless people immunised through vaccination (6.5%), compared to 40% of the general French population in 2000 [14]. High rates of HBsAg positivity have been reported among homeless people in the New Haven, USA (4.7%), while lower rates were observed in Iran (1.0%-2.6%), Canada (1.6%), Brazil (0.6%-3.3%), Colombia (0.4%) [13, 15-18].

The seroprevalence rate of HCV infection in Marseille homeless people (5.3%) was higher than that of the general local population whose anti-HCV antibodies prevalence decreased from 1.3% in 2004 [19] to 0.9% in 2015 [13]. High rates of positive anti-HCV antibodies were observed in many other homeless populations ranging from 2.2% to 36.2% [5,6] with the highest rate being reported in Dublin, Ireland, in 2005. The strongest independent determinant for anti-HCV seropositivity in Marseille homeless population was addiction to injected and inhaled drugs or the use of drug substitutes, followed by being over the age of 42 years, consuming cannabis and being underweight. An association between injected and non-injected drug use and HCV infection has been already reported in homeless populations [20]. By contrast, there was no association between cannabis consumption and HCV infection in these previous reports [20, 21], although such an association has been reported in the general population [22,23]. Although higher than in the overall French population [24], the proportion of homeless individuals reporting drug injection in our survey (2.1%) was lower that reported among other homeless populations [25, 26].

We found a high prevalence of previous exposure to *T. pallidum* (6.8%) including recent or ongoing syphilis infection (1.0%) in Marseille homeless population. No data on the sero-prevalence of syphilis is available regarding the general French population; however, a 35% increase in early syphilis cases was observed in 2014-2015 with the vast majority presenting among men who have sex with men [27]. High rates of syphilis infections, ranging from 0.3 to 33.2%, were reported in homeless populations from Brazil, Iran and England [8-10]. Similar to HBV infection, we identified Asian and sub-Saharan African origins as being independent risk factors for syphilis infection.

The strength of our study was that it confirmed active infection through detection and genotyping of HBV and HCV nucleotides. Identifying the sub-genotype and predictive aa mutations associated with drug resistance is of critical importance as these mutations are associated with transmission, response to therapy and treatment outcome. We showed a high genetic diversity of HBV but a low congruence between the origin of homeless individuals and that of the sequences most similar to that of their viruses (seen in one case of a Tunisian individual, the sequence was from the same origin). The data on HBV genotypes are in agreement with previous studies conducted in several African countries, showing that HBV-E is the most prevalent serotype circulating in western sub-Saharan Africa including Senegal, Burkina Faso, Nigeria [28]. The HBV-D (sub-genotype D1-D4) is prevalent worldwide and is predominant in Mediterranean area and in the Middle East [29], and HBV-A is spread equally across Europe and Africa. For HCV-1, a genotype known to be the most common in all anti-HCV infections and to be spread globally [30,31] accounted for four of our six sequences. HCV-3, predominately described in Asia, Pakistan and India, and HCV-4 which was principally reported in North Africa and the Middle East [31] were identified in two of our patients. A match between the origin of homeless individuals and that of the most similar sequences was found in five of six people (exception for sequence Seq5).

The only L31M substitution found in one HCV reverse transcriptase sequence in this study has been shown to confer resistance to the anti-HCV drug Elbasvir, Ledipasvir and to reduced susceptibility to Daclatasvir, which is widely available in many countries such as the USA, Japan and Tunisia [12]. Such resistance was demonstrated in treatment-naïve patients infected with HCV-1b, the sequence of which was close to our result.

Our study has some limitations. The bias or measurement errors can be subjectively created because of self-reported data. In questionnaires, no question addressed sexually risky behaviours.

Notwithstanding these limitations, in this paper we summarise our surveillance work over fifteen years. We evidenced a high prevalence of sexually transmitted infections in homeless people that remained relatively constant over the time period of the study. Findings such as this argue the case for systematic screening among this at-risk population and effective prevention programmes including health education activities, the provision of condoms and vaccination against HBV infection where appropriate.

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Potential conflicts of interest

No reported conflicts of interest.

Author Contributions Statement

TD, PC and PG contributed to experimental design, data analysis, statistics, interpretation and writing. VT, TL, ML, SB, HTD, PB administered questionnaires, examined patients and collected samples. VT, ML provided technical assistance. PC contributed to critically reviewing the manuscript. PG coordinated the work.

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Table 1. Univariate analysis of seropositivity result of each blood borne disease as a dependent variable.

Parameters	Total N (%)	HBsAg positivity		Anti-HCV seropositivity		Anti-*Treponema pallidum* seropositivity	
		n(%)	OR [95%CI], p-value	n(%)	OR [95%CI], p-value	n(%)	OR [95%CI], p-value
Total	**1890**	**54 (4.1)**		**90 (5.3)**		**106 (6.8)**	
Year of recruitment (1890) [1]			N/A		N/A		N/A
2000	261 (13.8)	9 (3.5)		22(8.8)		18 (6.9)	
2001	165 (8.73)	N/A		7 (4.6)		13 (7.9)	
2002	291 (15.4)	12 (5.0)		10 (3.5)		11 (3.8)	
2003	198 (10.5)	N/A		9 (4.6)		12 (6.1)	
2005	213 (10.3)	8 (4.0)		11 (5.5)		6 (3.2)	
2006	147 (7.8)	1 (0.8)		8 (6.4)		N/A	
2010	149 (7.9)	10 (12.2)		4 (5.0)		9 (12.0)	
2011	162 (8.5)	4 (2.9)		9 (6.5)		26 (18.9)	
2013	182 (9.6)	7 (4.5)		6 (3.8)		5 (3.2)	
2015	122 (6.5)	3 (3.2)		4 (4.2)		6 (6.4)	
Shelter (1890)							
B	816 (43.2)	25 (4.6)	REF	45 (6.0)	REF	41 (6.0)	REF
A	1074 (56.8)	29 (3.8)	0.83 [0.48-1.44], 0.5	45 (4.9)	0.78 [0.51-1.20], 0.26	65 (7.4)	1.24 [0.83-1.87], 0.28
Genre (1869)			N/A		N/A		N/A
Female	90 (4.8)	8 (12.9)		3 (4.0)		9 (11.7)	
Male	1779 (95.2)	46 (3.7)		87 (5.4)		97 (6.6)	
Age (1869)							
Mean age (±SD) (years)	43.1±14.6						
Age range (years)[2]	18-86						
≤42 years of age	959 (51.3)	33 (4.7)	REF	41 (4.7)	REF	34 (4.2)	REF
>42 years of age	910 (48.7)	21 (3.5)	0.73 [0.42-1.27], 0.27	49 (6.1)	1.32 [0.86-2.01], 0.19	71 (9.7)	**2.47 [1.62-3.8], <0.001**
Birthplace (1877)							
Europe	873 (46.5)	23 (4.0)	REF	53 (6.7)	REF	32 (4.4)	REF
North-Africa	853 (45.5)	10 (3.2)	0.79 [0.43-1.47], 0.47	28 (3.7)	**0.53 [0.33-0.86], 0.01**	56 (8.0)	**1.91 [1.22-3.00], 0.004**
Sub-Saharan Africa	115 (6.1)	9 (9.0)	**2.4 [1.04-5.55], 0.04**	6 (5.9)	0.87 [0.37-2.08], 0.76	10 (11.2)	**2.77 [1.31-5.7], 0.007**
Asia	32 (1.7)	4 (17.4)	**5.1 [1.61-16.3], 0.006**	2 (7.2)	1.07 [0.24-4.65], 0.92	7 (25.3)	**7.67 [3.0-19.5], <0.001**
America	4 (0.2)	0	N/A	0	N/A	1 (25)	N/A
Mean duration of residence in France for	12.9 (0-9.5)					N/A	

migrants (SD) (years) (554)							
Range of duration of residence in France for migrants (years)[2]	0-65					N/A	
≤ 42 months	277 (50.0)	13 (5.5)	REF	6 (2.6)	REF	13 (5.6)	REF
> 42 months	277 (50.0)	7 (3.3)	0.58 [0.22-1.49], 0.26	10 (4.7)	1.90 [0.68-5.31], 0.22	25 (12.3)	**2.34 [1.16-4.17], 0.02**
Mean duration of homelessness (SD), min, max (years)[2] (1795)	3.9 (0-29.7)						
Range of duration of homelessness (years)[2]	0-57						
≤ 1 year	992 (55.2)	34 (4.7)	REF	37 (4.1)	REF	44 (5.2)	REF
> 1 year	803 (44.7)	18 (3.4)	0.7 [0.4-1.25], 0.23	48 (6.8)	**1.7 [1.09-2.64], 0.018**	55 (8.6)	**1.71 [1.13-2.57], 0.01**
Alcohol consumption (1708)							
Rare or never	1056 (61.9)	41 (5.5)	REF	35 (3.8)	REF	56 (6.1)	REF
Frequent	652 (38.1)	11 (2.7)	**0.48 [0.24-0.94], 0.034**	46 (7.6)	**2.07 [1.31-3.26], 0.002**	49 (8.0)	1.33 [0.89-1.98], 0.16
Smoking cigarettes (1718)							
Never	372 (21.6)	15 (5.4)	REF	10 (3.2)	REF	21 (6.7)	REF
Yes	1346 (78.4)	37 (4.2)	0.75 [0.41-1.40], 0.37	71 (5.8)	1.82 [0.93-3.59], 0.08	84 (6.8)	1.02 [0.62-1.66], 0.95
Cannabis (marijuana) (610)							
Never	495 (81.1)	21 (5.7)	REF	10 (2.7)	REF	36 (9.9)	REF
Yes	115 (18.9)	3 (3.0)	0.52 [0.15-1.76], 0.3	13 (13.1)	**5.41 [2.29-12.75], p<0.001**	9 (9.2)	0.91 [0.42-1.97], 0.8
Drug addiction (771)							
Never	724 (93.9)	27 (5.0)	REF	25 (4.3)	REF	46 (8.1)	REF
Yes	47 (6.1)	1 (3.2)	0.69 [0.09-5.27], 0.72	10 (26.3)	**8.03 [3.51-18.33], p<0.001**	2 (5.3)	0.63 [0.15-2.70], 0.53
Injecting illicit substances	16 (2.1)	0	N/A	4 (36.4)	N/A	2 (18.1)	N/A
Snorting illicit substances	27 (3.5)	0	N/A	6 (27.3)	N/A	1 (4.5)	N/A
Using opioid	22 (2.9)	1 (7.1)	N/A	8 (44.5)	N/A	1 (7.3)	N/A

agonist treatment							
Blood transfusion (Yes versus No) (102)	102 (13.5)	7 (9.0)	2.2 [0.9-5.34], 0.08	7(9.0)	2.1 [0.88-5.07], 0.1	8 (10.7)	1.51 [0.68-3.39], 0.3
Before 1992	13 (1.7)	2 (20.0)	5.1 [1.05-25.4], 0.044[3]	3(30)	8.84 [2.17-36.04], 0.002[3]	1(10.0)	N/A
In and after 1992	89 (11.8)	5(5.6)	REF	4(4.5)	REF	7 (7.9)	N/A
BMI (1170) [4]							
Mean BMI (SD) (kg/m2)	23.5±4.0						
Range of BMI (kg/m2)	(13.5-50)						
Normal weight	743 (63.5)	28 (5.2)	REF	35 (5.3)	REF	34 (5.3)	REF
Underweight	75 (6.4)	1 (1.8)	0.32 [0.04-2.42], 0.27	6 (8.8)	1.74 [0.71-4.31], 0.23	4 (6.1)	1.17 [0.40-3.38], 0.78
Overweight	278 (23.8)	8 (4.0)	0.75 [0.33-1.68], 0.49	2 (0.8)	**0.15 [0.03-0.64], 0.01**	25 (10.6)	**2.12 [1.23-3.6], 0.006**
Obesity	74 (6.3)	2 (4.0)	0.75 [0.17-3.25], 0.7	0	N/A	1 (1.6)	0.30 [0.04-2.19], 0.23

Abbreviations: SD, standard deviation; BMI, Body mass index; N/A, not applicable; REF, Reference category.

[1]Number of individuals for whom data was available.

[2]Median of the variable is used for analysis.

[3]Low prevalence was not entered into multivariate model

[4]Based on the WHO classification, "underweight" is defined as having a body mass index (BMI) below 18.5, "normal" corresponds to a BMI between 18.5 and 25, "overweight" corresponds to a BMI ≥25, and "obese" refers to those with a BMI ≥30. Source: WHO. Body mass index – BMI http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/ahealthy-lifestyle/body-mass-index-bmi; (2019).

Bold lines indicate the variables recruited in initial multivariate model.

Table 2. Parameter estimates for the final weighted model for HBsAg, anti-HCV, and anti-*T. pallidum* antibodies

Parameter	HBsAg		anti-HCV		anti-*T. pallidum*	
	aOR [95%CI],	p-value	aOR [95%CI],	p-value	aOR [95%CI],	p-value
Age ≥42 years vs others	-		5.59 [1.62-19.3]	0.006	-	
Toxicomania	-		22.6 [5.35-95]	<0.001	-	
Cannabis	-		5.10 [1.59-16.38]	0.006	-	
BMI (underweight vs normal)	-		4.74 [1.18-18.94]	0.028	-	
Birthplace (Asian vs Europe)	5.12 [1.61-16.3]	0.006	-		7.67 [3.02-19.5]	<0.001
Birthplace (Sub-Saharan Africa vs Europe)	2.40 [1.05-5.56]	0.04	-		2.77 [1.31-5.86]	0.004

Abbreviations: aOR: adjusted odds ratio; CI: confidence interval; HBsAg: hepatitis B surface antigens, anti-HCV, antibodies to hepatitis C virus, anti-*T. pallidum*, antibodies to *Treponema pallidum*.

Table 3. Prevalence of serological blood-borne pathogens

Blood-borne pathogens	N (%)
HBV (CMIA test)	
HBsAg	54/1310 (4.1)
Anti-HBs	301/1312 (22.9)
Anti-HBc	379/1059 (35.5)
Combinations of HBV serologic markers	
Absence of immunity (HBsAg [neg], anti-HBc [neg], anti-HBs [neg])	557/1059 (52.6)
Immune due to vaccination (HBsAg [neg], anti-HBc [neg], anti-HBs [pos])	69/1059 (6.5)
Immune due to natural infection (HBsAg [neg], anti-HBc [pos], anti-HBs [pos])	190/1059 (17.9)
Recently resolved infection or inactive chronic infection (HBsAg [neg], anti-HBc [pos], anti-HBs [neg])	189/1059 (17.8)
Chronically or acutely infected (HBsAg [pos], anti-HBc [pos], anti-HBs [neg],)	54/1310 (4.1)
HCV (CMIA test)	90/1601 (5.3)
Treponema pallidum	
CMIA test	106/1564 (6.8)
RPR test	15/1564 (1.0)
Co-infection	
HBV+*T. pallidum* (CMIA test)	5/1156 (0.4)
HBV+HCV (CMIA test)	3/1298 (0.2)
HCV+ *T. pallidum* (CMIA test)	7/1538 (0.5)
HBV+HCV+ *T. pallidum* (CMIA test)	0/1145

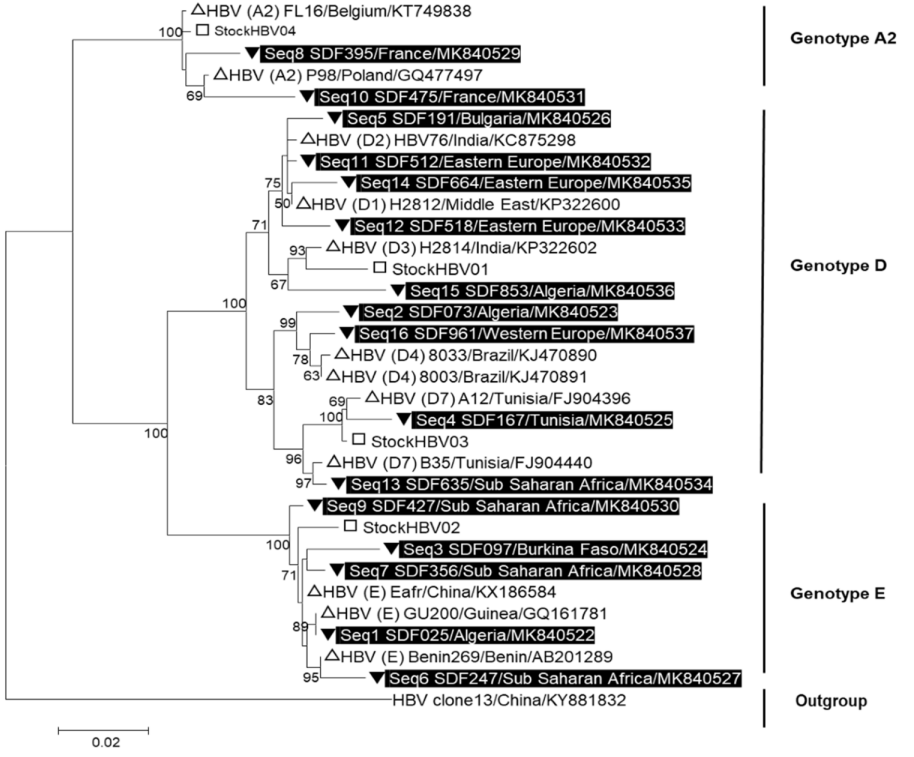
Abbreviations: CMIA; chemiluminescent microparticle immunoassay; HBsAg, hepatitis B surface antigen, anti-HBs, antibody to hepatitis B surface antigen; anti-HBc, anti-hepatitis B core total antibodies; antibodies to hepatitis C virus; anti-*T. pallidum*, antibody to *Treponema pallidum*.

Figure 1. Maximum-likelihood phylogenetic tree highlighting the position of the hepatitis B virus (HBV) identified in body lice of homeless people in Marseille (▼ and in black text highlight colour) compared to other HBV available in the GenBank database (△) or local reference sequences in Marseille general population (☐), and based on partial nt126-nt1213 polymerase (P) gene with HBV clone 13 (Gen Bank accession no. **NKY881832.1**) used as outgroup.

Note: ▼ Isolate name/country of origin/Gen Bank accession number
△ Sequence and patient code/ country of origin/Gen Bank accession number
☐ Sequence code in local sequence bank

Figure 2. Maximum-likelihood phylogenetic tree highlighting the position of the hepatitis C virus (HCV) identified in body lice of homeless people in Marseille in this study (▼, and in black text highlight colour), compared to their best match available in GenBank database (△) or reference sequences (○) recommended by https://hcv.geno2pheno.org/ or local reference sequences in Marseille population (☐), and based on partial 554-bp polyprotein (P).

Note: ▼ Isolate name/country of origin/Gen Bank accession number
△Sequence and patient code/ country of origin/Gen Bank accession number
○ Sequence and patient code/ country of origin/Gen Bank accession number
☐ Sequence code in local sequence bank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▼Seq11 SDF512/Eastern Europe/MK840532
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▼Seq12 SDF518/Eastern Europe/MK840533
△HBV (D3) H2814/India/KP322602
□ StockHBV01
▼Seq15 SDF853/Algeria/MK840536
▼Seq2 SDF073/Algeria/MK840523
▼Seq16 SDF961/Western Europe/MK840537
△HBV (D4) 8033/Brazil/KJ470890
△HBV (D4) 8003/Brazil/KJ470891
△HBV (D7) A12/Tunisia/FJ904396
▼Seq4 SDF167/Tunisia/MK840525
□ StockHBV03
△HBV (D7) B35/Tunisia/FJ904440
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▼Seq9 SDF427/Sub Saharan Africa/MK840530
□ StockHBV02
▼Seq3 SDF097/Burkina Faso/MK840524
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▼Seq6 SDF247/Sub Saharan Africa/MK840527
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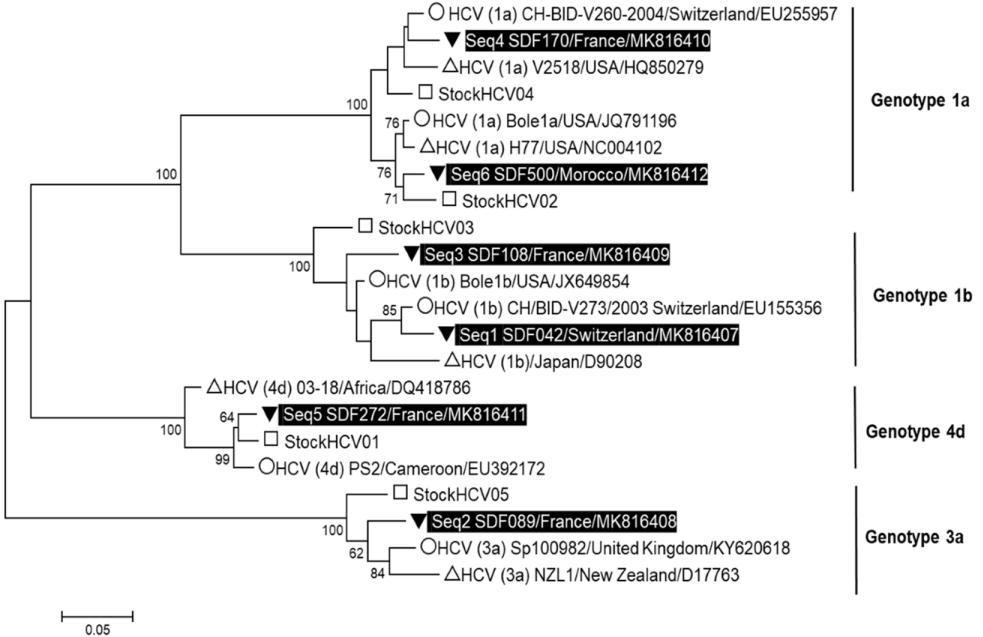
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Genotype 4d
Genotype 3a
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