Universidade Nova de Lisboa
Instituto de Higiene e Medicina Tropical

Sampling methods to reach hard populations: appraising and comparing different statistical methods with an application to an HIV prevalence study

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I) Barros, A. B., Dias, S. F., & Martins, M. R. O. (2015). Hard-to-reach populations of men who have sex with men and sex workers: A systematic review on sampling methods. Systematic Reviews, 4(1). https://doi.org/10.1186/s13643-015-0129-9

II) Barros, A. B., & Martins, M. R. O. (2020). Improving underestimation of HIV prevalence in surveys using Time-Location Sampling. Journal of Urban Health: Bulletin of the New York Academy of Medicine. https://doi.org/10.1007/s11524-019-00415-8 (online published)

III) Barros, A. B., & Martins, M. R. O. (2020). Calibration of HIV prevalence estimates in most-at-risk populations: results from a two countries simulation study (to be submitted)

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If we knew what it was we were doing, it would not be called research, would it?

Albert Einstein

Resumo

Os dados de saúde, e especialmente os relacionados com o VIH, são menos robustos para populações de difícil acesso, também chamadas populações-chave ou populações de maior risco, do que para a população em geral e tendem a ser subestimados devido principalmente a problemas relacionados com estigma, discriminação e complexidade na amostragem dos elementos da população. O estigma e a discriminação desencorajam as populações-chave e, especialmente, os indivíduos VIH positivos a frequentar os serviços de saúde, a participarem em inquéritos e a revelarem os seus comportamentos de risco. As complexidades da amostragem ocorrem porque nos países onde essas populações são discriminadas, elas tendem a permanecer ocultas e, consequentemente, é mais difícil identificá-las e recolher informação. Ou seja, não é possível garantir amostras representativas dos elementos da população. O uso de estratégias de amostragem não probabilística tem sido o mais utilizado para rastrear as populações-chave mas os resultados obtidos correm o risco de serem enviesados, o que significa que, por exemplo, as estimativas de prevalência de VIH obtidas para essas populações podem não ser precisas. Nos últimos anos, no entanto, vários métodos não probabilísticos foram utilizados. Extensões desses métodos foram desenvolvidas para evitar que os elementos amostrados sejam escolhidos de maneira casual, o que deu origem aos métodos semi-probabilísticos. Focando num dos métodos semi-probabilísticos mais utilizados, o método de amostragem local-tempo (TLS), desenvolvemos uma abordagem para melhorar a precisão das estimativas de prevalência do VIH. O novo método, chamado CARES, que significa calibração pelos resíduos, consiste em imputar pesos aos entrevistados, considerando o percentil ao qual seus resíduos de regressão logística pertencem. Usando duas bases de dados de HSH de Portugal e Espanha, começámos por as ajustar o mais próximo possível de universos TLS e dos quais várias amostras TLS foram simuladas. Para cada amostra simulada, foi registada a prevalência do VIH, não ponderada e ponderada pelos pesos amostrais. Um modelo de regressão logística foi aplicado e os resíduos foram registados. O método CARES foi aplicado e a prevalência do VIH foi novamente calculada. A prevalência estimada de VIH obtida pelo método CARES foi comparada com as estimativas de prevalência calculadas usando apenas o método de amostragem TLS. Os resultados mostraram que o método CARES melhora as estimativas de prevalência do VIH obtidas quando o método de amostragem local-tempo é usado para recrutar os entrevistados. Este método é uma nova abordagem que visa fornecer melhores estimativas de prevalência do VIH e pode ser muito útil sempre que técnicas de amostragem mais confiáveis não possam ser aplicadas.

Palavras-Chave: VIH, Regressão Logística, Populações-chave, Resíduos, Amostragem tempo-local

Abstract

Health data and especially HIV related data is less robust for hard-to-reach populations, also called key-populations or most-at-risk populations, than for general population and tend to be underestimated mainly due to issues related to stigma, discrimination and complexities in sampling the population elements. Stigma and discrimination discourages key-populations and specially HIV positive individuals to frequent health care facilities, take part in surveys and reveal their risk behaviours. Complexities in sampling happen because in countries where these populations are discriminated they tend to remain hidden and consequently it is more difficult to identify them and collect data. Therefore, it is not possible to assure representative samplings of the population elements. The use of non-probability sampling strategies have been the most used to screen key-populations but results obtained have the risk of being biased, this means that, for instance, HIV prevalence estimates obtained for these populations might not be accurate. In the last years however, several non-probabilistic methods have been used. Extensions of those methods have been developed in order to avoid that the sampled elements are chosen in a casual way, which gave rise to the semi-probabilistic methods. Focusing on one of the most used semi-probabilistic methods, the time-location sampling method (TLS), we developed an approach to improve accuracy of HIV prevalence estimates. The new method, called CARES, which means Calibration on Residuals, consists in imputing weights to respondents, considering the percentile to which their logistic regression residues belong. Using two MSM databases from Portugal and Spain, we began by adjusting the databases as close as possible to a TLS universe and from which several TLS samples were drawn. For each simulated sample HIV prevalence, unweighted and weighted by the sampling weights was recorded. A logistic regression model was run and residues were recorded. The CARES method was applied and HIV prevalence was again calculated. The estimated HIV prevalence obtained by CARES method was compared to HIV prevalence estimates calculated using the TLS method only. Results showed that CARES method improves HIV prevalence estimates obtained when time location sampling method is used to recruit respondents. This method is a new approach that aims to provide better HIV prevalence estimates and might be very useful whenever more reliable sampling techniques cannot be applied.

Key-words: HIV, Logistic Regression, Key-populations, Residues, Time-location sampling

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Acronyms

AIDS	Acquired Immunodeficiency Syndrome
CARES	Calibration on Residuals
CI	Confidence Interval
EMIS	European MSM Internet Survey
FSW	Female Sex Worker
HIV	Human Immunodeficiency Virus
HRP	Hard-To-Reach Population
HSH	Homens Que Praticam Sexo Com Homens
IDU	Injecting Drug Users
ILGA	International Lesbian and Gay Association
LCI	Lower Confidence Interval
MARP	Most At Risk Population
MSM	Men Who Have Sex With Men
MSW	Male Sex Worker
PLWHA	People Living With HIV/AIDS
PWID	People Who Inject Drugs
PEP	Pre-Exposure Prophylaxis
RDS	Respondent-Driven Sampling
SE	Standard Error
STI	Sexually Transmitted Infection
SW	Sex Worker
TG	Transgender
TGW	Transgender Women
TGSW	Transgender Women Sex Worker
TLS	Time-Location Sampling
UAI	Unprotected Anal Intercourse
UNAIDS	Joint United Nations Program On HIV/AIDS
UCI	Upper Confidence Interval
VBS	Venue Based Sampling
VDT	Venue Day Time
VIH	Vírus da Imunodeficiência Humana
WHO	World Health Organization

Part I – INTRODUCTION

INTRODUCTION

1 Hard-to-reach populations and the burden of HIV

1.1 Preamble

It is estimated that in 2018 about 37.9 million [32.7 million–44 million] people were living with HIV in the world (UNAIDS, 2019b). Despite new infections have decreased about 40% since the peak in 1997, about 1.7 million [1.4 million–2.3 million] became newly infected in 2018 (UNAIDS, 2019b). The vast majority of persons infected with HIV live in low and middle-income countries and about 1 million people die every year with HIV-related causes in the sub-Saharan Africa (Frank et al., 2019).

International Health seeks to identify, develop and implement practices and policies aiming to improve health and well-being of the most disadvantaged and vulnerable people in the world (WHO, 2018). International Health is population-based priority, mainly the poorer and vulnerable, and focus on preventive measures. It is a multidisciplinary and interdisciplinary approach with an emphasis on health as a public good (Koplan et al., 2009).

The World Health Organisation (WHO) considers some sub-populations at more risk of HIV infection than the general population (WHO, 2016). Men who have sex with men (MSM), sex workers (SW), transgender people (TG) and people who inject drugs (IDU) are some of the sub-population groups that are particularly vulnerable to HIV and frequently lack adequate access to services (UNAIDS, 2018c).

These sub-populations, also called key-populations, most-at-risk populations (MARPs) or hard-to-reach populations (HRP) are defined as populations at an increased risk for HIV irrespective of the epidemic type or local context (WHO, 2016) and are often associated with illegal or socially stigmatized behaviours (WHO, 2016).

In 2019, key-populations and their sexual partners accounted for about 54% of new infections in the world, accounting for 95% of new HIV infections in Middle East and North Africa, 65% in Latin America, 47% in the Caribbean (UNAIDS, 2019b).

According to UNAIDS estimates MSM have 27 times more risk to become HIV positive than the general population (AVERT, 2019a), IDU have 22 times more risk to become HIV positive than the general population (AVERT, 2019b) , sex workers (SW) have 13 times more risk of acquiring HIV than the general population (AVERT, 2018a), transgender persons have 49 times more risk of acquiring HIV than the general population (AVERT, 2018b).

Further, some subgroups of key-populations may have higher risk for HIV infection than others. For example, a study in Nigeria of young FSW found that HIV prevalence was higher among brothel based FSWs compared to non-brothel based FSWs (Okafor, Crutzen, Ifeanyi, Adebajo, & Van den Borne, 2017).

It is not uncommon that people from one key population also belongs to another key population and therefore engage in more than one high risk behaviour, for instance FSWs who also are IDUs. Such persons are likely to have higher HIV prevalence rates than those with only one type of risk (WHO, 2016).

A brief description and the burden of HIV on sex workers is presented in the next sub-chapter, followed by injection drug users, and men who have sex with men. This chapter ends with the description and the burden of HIV on transgender people.

1.2 Sex workers

Sex workers are persons (female, male or transgender) who exchange sex for money or goods, either regularly or occasionally (WHO, 2015a). It is estimated that the average HIV prevalence among SW in the world is approximately 9% (AVERT, 2018a) however there is a large variation within countries, regions and sub-populations. For example, in Nigeria the HIV prevalence is 24.5% (compared with 3.7% among the general population ages 15–49 years), in Latvia is 22.2% (compared with 0.7% in the general population), and in Rwanda is 50.8% (compared with 2.9% in the general population) (WHO, 2016).

HIV prevalence among FSW in eastern and southern Africa is very high. In Lesotho, Malawi, South Africa and Zimbabwe more than 50% of FSW are living with HIV (UNAIDS, 2018c).

HIV prevalence is higher among male sex workers (MSW) than FSW and other MSM (Baral et al., 2015). In ten European countries, the median HIV prevalence among MSW was 8.9% also, data from five African countries presented a median HIV prevalence of 12.5% (Baral et al., 2015).

Transgender women sex workers (TGSW) have a disproportionate high risk for HIV infection compared with natal male and female sex workers; worldwide is estimated a HIV prevalence of 27.3% among TGSW (Poteat et al., 2015). Few data exist on sex work in transgender men, people assigned female at birth who identify or express themselves as men, therefore they are not included in this analysis.

Globally, SWs are at increased risk for HIV infection for several reasons: a) they are often stigmatized, criminalised and suffer from violence; even in less repressive countries the law hardly protects them (AVERT, 2018a); b) they have a high number of sexual partners and report an inconsistent condom use, often due to the clients' pressure (AVERT, 2018a); c) sometimes SW who injects drugs and share needles are stigmatized in the workplace and are forced to go to the streets where they are more exposed to violence and are more vulnerable to arrests and extortion (Rusakova, Rakhmetova, & Strathdee, 2015); d) some migrants may become SW if they cannot find a job or other mean of subsistence; migrant SW are frequently arrested, specially if they live abroad and do not have immigration status (Goldenberg, Brouwer, Rocha Jimenez, Morales Miranda, & Rivera Mindt, 2016).

1.3 Injection drug users

Injection drug users (IDU), or people who injects drugs (PWID), refers to people who inject in the bloodstream psychotropic or psychoactive substances (for example: opioids, amphetamines-type stimulants, cocaine) for non-medical purposes (WHO, 2016). WHO estimates that around 13 million people inject drugs worldwide and 1.7 million of them are living with HIV (WHO, 2019). It is estimated that globally, IDUs are 22 times more likely to be HIV positive than the rest of the population (UNAIDS, 2018c). IDUs account for 10% of HIV infections globally and 30% of those are outside of Africa (WHO, 2019). Regional HIV prevalence rates are high in people who inject drugs in all parts of the world. For example, in Pakistan HIV prevalence in IDUs is 21%

(compared with 0.1% in the general population ages 15–49 years) (UNAIDS, 2018a), in Indonesia HIV prevalence in IDUs is 29% (compared with 0.4% in the general population ages 15–49 years) (UNAIDS, 2018a) and in Portugal HIV prevalence in IDUs is 21.3% (compared with 0.5% in the general population ages 15–49 years)(UNAIDS, 2018a).

Globally, IDUs are at increased risk for HIV infection for several reasons: a) sharing needles: if an HIV-positive person shares needles, the infected blood can be injected in the next person; sterile syringes are not always available also, the lack of awareness or education about safe injecting is another major reason for sharing needles (AVERT, 2019b); b) the criminalisation of people who use drugs leads to more risky forms of drug use, reinforces the marginalisation and also discourages them from accessing harm reduction and other healthcare services (AVERT, 2019b), which increases their vulnerability to HIV infection and has a negative effect on HIV prevention and treatment (DeBeck et al., 2017); c) most drug users and IDUs particularly are considered poor (AVERT, 2019b), social and economic disadvantage is strongly associated with drug use disorder (UNODC, 2017) which means that people choose cheaper ways of taking drugs such as sharing needles (AVERT, 2019b). Poverty may also lead to behaviours associated with increased risk for HIV such as sex work or selling sex for drugs; d) IDUs who are SWs have higher HIV prevalence than non SWs IDUs, and are more vulnerable to HIV infection (Campeau et al., 2018); e) Women IDU are more susceptible to HIV infection due to gender-based stigmas, namely inequitable gender power, economic pressures, and poor availability of needles and condoms (Mburu, Limmer, & Holland, 2019); additionally, women who also are IDUs are more likely to experience sexual violence from police and law enforcement agencies (UNAIDS, 2018c).

1.4 Men who have sex with men

Men who have sex with men (MSM) refer to men who engage in sexual/romantic activity with other men, regardless of how they identify themselves. Globally, MSM are 27 times more likely to become HIV positive than the rest of the population (UNAIDS, 2019b) and the epidemics continue to expand in most regions (UNAIDS, 2016). MSM accounts for about 12% of new HIV infections in western and central Africa, 20% of new infections in eastern and central Europe, more than 25% of new HIV infections in Asia and Pacific, 41% of new infections in Latin America, and 57% of new HIV infections in western and central Europe and North America (UNAIDS, 2018c). The prevalence of HIV infection in MSM is high, for example in Romania is 18.2% (compared with 0.1% in the general population ages 15–49 years), in Indonesia is 25% (compared with 0.4% in the general population ages 15–49 years), and in Mauritania, the country with the highest HIV prevalence among MSM in the world, is 44.4% (compared with 0.2% in the general population ages 15–49 years) (UNAIDS, 2018a).

Some countries have laws to protect against discrimination of homosexuality, such as Mexico, Nepal and Portugal (ILGA, 2019), others have made significant progresses by recognising same sex acts, like Brazil, Canada, Spain and Australia (ILGA, 2019). However, most African countries, some Latin America and Caribbean countries and Asian countries criminalise same sex consensual acts with severe penalties ranging from 1-20 years in prison (Lebanon and Malaysia respectively) until death penalty in countries such as Mauritania, Nigeria, Sudan in Africa and Afghanistan, Iran and Saudi Arabia in Asia (ILGA, 2019). In countries where laws criminalise same sex behaviours and countries where this population is stigmatized and discriminated, MSM tend to remain hidden and away from preventive healthcare plans, being therefore exposed to high risk of HIV infection (UNAIDS, 2019a).

Globally, MSMs are at increased risk for HIV infection, among other factors, because: a) biological factors - unprotected anal sex carries a higher risk of transmission than vaginal sex (UNAIDS, 2018c); also, MSM who have some sexually transmitted infection (STI) are more susceptible to HIV infection (Chow, Grulich, & Fairley, 2019); b) behavioural factors - inconsistent condom use, multiple partners, unawareness of the HIV status, also, alcohol and drug use can make them more likely to have unprotected sex and a higher number of sexual partners (AVERT, 2019a); HIV positive MSM may forget to take the ART medication and HIV negative men might miss taking the post-exposure prophylaxis (PEP) (Rodger et al., 2019), c) legal factors – in countries where same-sex relations are criminalised and in countries where there are no protective laws MSMs are less likely to have access to HIV related services (UNAIDS, 2019a); d) social and cultural factors – even in countries with less repressive laws is common that

MSMs experience stigma and discrimination, either inside their own families, friends, co-workers, etc. (Brooks, Landrian, Nieto, & Fehrenbacher, 2019) which leads them not to disclose their sexual identity and therefore not accessing health services; additionally, MSMs are likely to experience depression due to social isolation (Wagner et al., 2018), which makes them more vulnerable.

1.5 Transgender people

Transgender people is a term for people whose gender identity does not conform with the sex at birth and includes people who are transsexual, transgender or otherwise gender non-conforming (WHO, 2015b). Transgender people have diverse sexual orientation and behaviours.

Transgender-specific HIV data is limited. The majority of published literature focuses on transgender women given the documented heavy burden of HIV infection they bear (WHO, 2015b).

Estimates say that there are 25 million transgender people living in the world (Winter et al., 2016) and they are one of the key populations most affected by the HIV epidemic. Transgender people are about 49 times more likely to be HIV positive than the rest of adult population (AVERT, 2018b). Global estimates report an HIV prevalence among transgender woman of 19% (UNAIDS, 2014) however, this prevalence varies greatly with countries, for example Armenia, Cuba, Pakistan report HIV prevalence of 5% or less, Mexico and Malaysia report 8%-10% of HIV prevalence and Brazil, Panama and Jamaica report prevalences between 30%-51% of HIV (UNAIDS, 2018b). Data from Latin America and Caribbean show that transgender women sex workers have higher HIV prevalence than non-transgender male and female sex workers (UNAIDS, 2016)

Globally, transgender people are at increased risk for HIV infection for several factors. The most relevant are: a) social – transgender people face high levels of stigma, discrimination and abuse. This makes them to remain hidden and therefore less likely to access healthcare services and highly exposed to HIV (AVERT, 2018b); b) economic – transgender people are more likely to have less years of schooling, move away from family and friends, workplace discrimination and limited economic opportunities (Winter et al., 2016); c) legal – in countries where laws criminalise transgender people, they are less likely to have access to HIV related services, more likely to face discrimination and less likely to have economic opportunities (Winter et al., 2016); d) sex work – social, economic and legal factors leads transgender people to exclusion and economic vulnerability and for this reason sex work is the most viable form to get money (AVERT, 2018b). A high proportion of transgender people engage in sex work, for example is estimated that the percentage of transgender women who engage in sex work is 90% in India, 84% in Malaysia, 81% in Indonesia, 47% in El Salvador and 36% in Cambodia (UNAIDS, 2014); e) transition healthcare – high costs are associated with transition healthcare (for example gender-changing hormones) that can put extra pressure on transgender people to make money and to engage in higher risk sex (AVERT, 2018b).

2 Sampling methods to reach hard populations

2.1 Preamble

Health data obtained for key-populations, and especially HIV related data, is less robust than for general population (WHO, 2016) and tend to be underestimated (Prüss-Ustün et al., 2013) mainly due to 1) issues related to stigma and discrimination and 2) complexities in sampling the population-elements, because:

1) HIV positive individuals are less willing to take part in surveys (Fogel et al., 2019) and reveal their risk behaviours (Hladik, Benech, Bateganya, & Hakim, 2016); also, it is not uncommon find out that some elements of key-population ignore its serological status (Chandler CJ, Sang JM, Bukowski LA, Andrade E, Eaton LA, Stall RD, 2018) and some of them may have seroconverted since the last HIV test (Fogel et al., 2019).

2) Complexities in sampling happen because in countries where key-populations are discriminated is more difficult to identify them and collect data (WHO, 2016) and if key-populations tend to remain hidden, it is not possible to count how many they are. For this reason it is not possible to assure representative samplings of the population elements (Rao et al., 2017) therefore it is almost impossible the use of probability-sampling strategies to choose members at random to be screened.

In these circumstances, the use of non-probability sampling strategies are the most used to screen hard-to-reach populations.

Non-probability sampling encompasses a group of sampling strategies that selects the elements to be included in a study based on a subjective judgement. In non-probability sampling not all members of the population have the same chance of participate because the sample is restricted to a part of the population that is accessible (Cochran, 2007). Results obtained through this type of sample have the risk of being biased (that is, not being representative of the target population) (Jing Qin, 2017). In the last years however, several non-probabilistic methods have been more used and extensions of those methods have been developed. As a consequence of this development nowadays non-probabilistic methods are divided into two categories: a) non-probabilistic sampling methods - the sampled elements are chosen arbitrarily or casually. In these methods it is not possible to estimate the probability of each element being included in the sample and consequently there is no way of making inferences to the population (Magnani, Sabin, Saidel, & Heckathorn, 2005); b) semi-probabilistic sampling methods – the sampled elements are chosen not totally in a casual way, something similar to sampling frames are constructed and in theory these methods allow to determine the probability of each element being included in the sample (Krivitsky & Morris, 2017).

In the next sub-chapters are presented the most frequently used methods to survey hard-to-reach populations: the non-probabilistic methods of Convenience Sampling, Purposive Sampling, Snowball Sampling, and the semi-probabilistic methods of Internet Sampling, Respondent Driven Sampling (RDS) and Time Location Sampling (TLS).

2.2 Convenience sampling

Convenience sampling is a non-probabilistic, venue-based sampling technique where the respondents are selected only because they are available, accessible or other way. The fieldwork research usually starts by doing a formative research to identify venues (places) and times the key-population congregates (Abrams, 2010). Community organisations with access to the key-populations play an important role by helping researchers gain access to an otherwise even hard-to-reach group (Bonevski et al., 2014). After identifying the venues researchers may start the survey at their own convenience.

Convenience sampling is easy to apply, with few rules on how the sample should be collected (UNAIDS, 2011). The cost and time are small in comparison to other sampling techniques. It is an easy way to achieve the desired sample size in a relatively fast and inexpensive way (Schwarcz, Spindler, Scheer, Valleroy, & Lansky, 2007). Results are prone to bias because those who participate in the study may not be representative of the population nevertheless, public health often searches quick information from key-groups in order to rapidly address a problem therefore, reliance on convenience samples is likely to continue (Schwarcz et al., 2007).

In countries where key-populations are stigmatized and/or criminalized, sometimes the convenience sampling is the most secure method to reach them, such as MSM in Lebanon (Mutchler et al., 2018). Additionally, some sub-populations can hardly be identified and convenience sampling is the most easy, if not the only, way to access them. For example, a study conducted in the United States of America used the convenience sampling to interview young non-infected transgender women to access their awareness about Pre-Exposure Prophylaxis (PrEP) (Kuhns et al., 2016).

2.3 Purposive sampling

Purposive sampling, or judgement sampling, is a set of non-probabilistic methods where respondents are selected by the judgement of the researcher when choosing the elements to be included in the survey. It is a venue-based technique that does not need underlying theories or rules; after a formative research, the researcher chooses the persons that best represent a particular characteristic that is of interest to the study (Bernard, 2006).

There are several types of purposeful samplings each with a different purpose. The two most common are: a) the maximal variation sampling, in which diverse individuals are chosen and who are expected to hold different perspectives of the study subject for example, might be by race, gender, education, or other variables that would differentiate participants (Tolley EE, Ulin PR, Mack N, Robinson ET, 2016); b) the extreme case sampling, where the researcher selects individuals who provide especially unusual, troublesome, or enlightened information (Creswell & Plano Clark, 2018); in this case, the researcher might use homogeneous sampling by selecting individuals who belong to the same subgroup with distinctive characteristics (Tolley EE, Ulin PR, Mack N, Robinson ET, 2016).

Preconceived notions of the researchers or judgements that have not been based on clear criteria might influence the outcomes and therefore, results are more subject to ambiguity and bias (Guarte & Barrios, 2006). Sometimes the purposive sampling requires an extra-collaboration from others to identify participants matching characteristics sought which can be much time-consuming (Valerio et al., 2016).

Purposive sampling is especially useful when some sub-populations are even harder to reach than the key-population where they belong. A study conducted in a small Indonesian village, used a purposive sampling to select HIV positive MSM to access the relationship between family acceptance and quality of life and self-esteem (Putra IN, Waluyo A, 2019).

2.4 Snowball sampling

Snowball sampling is a non-probabilistic method used to survey respondents that are difficult to locate or identify. It is a chain-referral technique that relies on the assumption that peers are better to identify and recruit respondents from key-populations than the researchers (Sadler, Lee, Lim, & Fullerton, 2010). When using snowball sampling researchers must begin by identifying an initial number of respondents who will then help to identify other subgroup members to take part on the survey (Faugier & Sargeant, 1997). These other subgroup members, will, in turn, be asked to refer other individuals with the same characteristics as previously defined by researchers. The chain-referral process continues until the desired sample size is achieved and by this way researchers believe being able to reach the hard-to-reach target group (Sadler et al., 2010). Snowball sampling is a time and cost efficient way to recruit members of hidden populations (Kendall et al., 2008), especially those who have strong social networks (UNAIDS, 2011).

Initiating the process of finding respondents and start the chain-referral process may be very time consuming and labour intensive to acquire (Atkinson & Flint, 2001); the method of snowball sampling rests on the existence of social networks among individuals of the same population. However, some groups may consist of few isolated individuals whose social networks are near to none and those individuals hardly be included in the survey (Miller & Brewer, 2016). Therefore the existence of selection bias limits the validity of the sample (Miller & Brewer, 2016). Additional bias exists in snowball sampling method because selected elements are dependent on the subjective choices of initially accessed respondents (Atkinson & Flint, 2001), and also because most people tend to name acquaintances who are similar to themselves demographically: by race, ethnicity, education, income or religion (UNAIDS, 2011).

Snowball sampling is useful to recruit populations who usually have strong social networks such as the MSM community. A study conducted in Nigeria, applied the snowball sampling method to access the HIV prevalence and risky sexual behaviour in a MSM community (Ekpenyong et al., 2019).

2.5 Internet sampling

Internet sampling is a semi-probabilistic method used to sample, not only but also, key-populations at risk of HIV. The fast development of new technologies gave more and more people worldwide access to the Internet. Recent estimates show that 57% of the world population have access to the internet, with a penetration rate ranging from 40% in Africa until 90% in North America (IWS, 2019) and for this reason Internet is an invaluable tool through which one can search and give information (Miller RL, Brewer JD, 2003). Additionally, Internet is the world most extensive venue for sexual information and sex negotiation (Pequegnat et al., 2007) and therefore to recruit key-populations at increased risk for HIV and hard to access such as, for example, young MSM and sexual minorities (Noble, Jones, Bowles, DiNenno, & Tregear, 2017). Recruiting respondents through the Internet is an efficient way to reach a large number of people in short time, has lower costs and greater level of confidentiality when compared to other recruitment methods (Rosser et al., 2011).

Internet sampling can be considered a semi-probabilistic method because, under some circumstances is possible to know how many persons are visiting a webpage, which means the existence of a sampling frame (Hays, Liu, & Kapteyn, 2015). There are several ways of conducting internet surveys: internet convenience sampling (Hays et al., 2015), internet snowball sampling (Baltar & Brunet, 2012) and internet respondent-driven sampling (Strömdahl, Lu, Bengtsson, Liljeros, & Thorson, 2015).

Internet-based studies are subject to several limitations such as selection bias, because only those who have internet access can participate (Rosser et al., 2011). Additionally bias exists if selected elements belong to subgroups that engage in higher risk behaviours associated with internet (Pequegnat et al., 2007). Some studies also suggest that internet-based studies recruit elements whose socio-demographic characteristics differ from the key population they belong and therefore may not be representative (Noble et al., 2017).

The European MSM Internet Survey (EMIS) is the largest transnational survey among MSM ever conducted in terms of the number of participants and number of countries covered. In 2010 collected data from about 170,000 MSM from 38 countries (The EMIS Network, 2013). In 2017 a second version of EMIS was conducted in 50 countries and more than 100,000 men in the European Union took part, in addition to more than 6,000 in EFTA countries, and about 7,000 in countries in the EU enlargement area (Turkey, North Macedonia, Albania, Montenegro and Serbia) or in the European Neighbourhood Policy (Algeria, Morocco, Egypt, Israel, Jordan, Lebanon, Libya, Palestine, Syria, Tunisia in the South and Armenia, Azerbaijan, Belarus, Georgia, Moldova, Ukraine), and 6,000 MSM in Russia, 6,000 in Canada, and 3,500 in the Philippines (ESTICOM Project, 2018).

2.6 Respondent driven sampling

Respondent driven sampling (RDS) is a semi-probabilistic method that became very popular due to its cost-effectiveness in reaching hard populations at risk of HIV (Li et al., 2018). The method is similar to snowball sampling in that involves chain referral sampling but has two main innovations. First, the methods' recruitment technique relies on respondents of each wave to recruit the next wave by distributing uniquely identified coupons to others; through many waves, the dependence of the final sample on the initial sample is reduced (Gile, 2011). The second innovation is related to the process of weighting the data after the sample is collected; respondents are asked to estimate how many people they know in the hidden population and based on this information some post-stratification procedures are applied (Mouw & Verdery, 2012).

As a sampling procedure, RDS begins with a convenience sample of participants, known as seeds, who receive a fixed number of coupons (usually 3) to pass on to their peers who will be the sample' "first wave" (Li et al., 2018). When the first wave of eligible peers enters the study, they receive the same number of coupons each to recruit other peers. The process continues until the desired sample size or time period is reached (Gile, Beaudry, Handcock, & Ott, 2018). When the recruitment is completed, respondents return to receive the rewards for having recruited peers and to do a "post-interview" focusing on recruitment factors (Shi, Cameron, & Heckathorn, 2019).

RDS is a semi-probabilistic method because provides a means for drawing probability samples of populations when some assumptions about the structure of the network that links sample to the population are satisfied (Shi et al., 2019). However, several of those assumptions are not easy to meet and therefore RDS suffers from sampling bias (Li et al., 2018). Some sources of bias include nonresponse, recruitment effectiveness (some groups are better recruiters than others) (Gile et al., 2018) and recruiters homophily' tendency (recruiters tend to recruit from their in-group, specifically, positives recruiting positives) (Shi et al., 2019). Therefore, the accuracy of RDS estimates is sensitive to the assumptions made about the social network of the key population in study (Mouw & Verdery, 2012).

The RDS method as a recruitment technique to reach hard populations is one of the most used nowadays, they provide major organizations with data for key funding and programmatic decisions (e.g. UNAIDS, WHO) therefore they must be properly planned and implemented (Johnston, Chen, Silva-Santisteban, & Raymond, 2013).

More and more studies are using the RDS to recruit respondents. For example a study conducted in Brazil, the second national Biological and Behavioural Surveillance Survey (BBSS) of HIV, syphilis, and hepatitis B and C among MSM, used RDS method (Kendall et al., 2019).

2.7 Time location sampling

Time-location sampling (TLS), or venue-based sampling (VBS), is a semi-probabilistic method used to recruit populations that are hard to reach. It is a venue-based method that has its origin in the 80's on the targeted sampling that recruited hard-to-reach respondents at physical venues ensuring by this way their inclusion on the survey (Watters & Biernacki, 1989).

TLS has three main phases: 1) the formative assessment, 2) defining the universe and the sampling frame and 3) the data collection (MacKellar et al., 2007; Raymond, Ick, Grasso, Vaudrey, & McFarland, 2010; Semaan, 2010).

1) In the formative assessment researchers define ways of accessing the key population, map the physical venues where the key population congregates and interview key informants. Formative research should be rigorous to produce detailed information on the topic and/or the key population. Interview with key informants may provide additional unknown information about the key population. Key informants should be well informed about some relevant aspects of the key

population; ideally they must offer insights into the context of HIV risk behaviour and types of venues where the respondents can be recruited. At this stage, researchers collect information concerning the attendance flow of the key-population; on each venue they observe the activity and count the number of clients entering the venue. This is done to confirm that the key-population attends the venue, to know what are the days/times of high attendance and how many persons attend during these times. This is called the type I enumeration method and is designed to collect quantitative data that will support the inclusion of a venue/days time-periods (VDTs) in the universe of venues.

2) In the second phase a list of eligible venues, days and time-periods (VDTs) is constructed. The universe of eligible venues can be any public or private locations attended by the key-population, except those venues that specifically serve HIV positive members because they would artificially increase the representation of HIV positive individuals in the final sample. Although all venues can be included in the universe of venues, not all venues will be included in the sampling frame because of safety concerns, low levels of attendance, disapproval by owners or managers, etc. Venues can have different VDTs and if the daytime period is large, it can be broken into 4 hours' period for that venue. At this stage, the type II enumeration method is applied which consists in counting the number of eligible persons who attend a venue at a specific day and time-period; this is especially useful to estimate the potential number of interviews a VDT can generate during a sampling event.

3) After defining the sampling frame, researchers randomly select a sample of venues and VDTs to collect the data. During this phase, ideally a two-stage sampling should be applied. In the first stage, a simple random sample of the VDTs, preferably with a probability proportional to the number of the eligible persons at each VDT should be selected. On the second phase a random or systematic sample to select potential respondents and conduct the survey should be applied. If possible, relevant data should also be collected on those who refuse to participate in the survey. At this stage the type III enumeration method is applied which consists of counting how many potential eligible participants enter the venue during the interview phase.

TLS is a semi-probabilistic method because allows to construct a sampling frame from which to choose a sample of venues and VDTs, collect relevant data to calculate weights to control for unequal probability of selection (Armstrong et al., 2015; Wei et al., 2016). Nevertheless, methods proposed to control for unequal probabilities of selection are not consensual and there is a continuum of proposals about how to get the best estimates (Gustafson et al., 2013; J. M. Karon & Wejnert, 2012; Leon, Jauffret-Roustide, & Le Strat, 2015; Sommen et al., 2018). Collect data to calculate weights can be cumbersome and originate imprecise replies which might lead to biases results (Kalton, 2014; J. Karon & Wejnert, 2014). Results obtained using TLS also suffer from additional bias. Some key populations are very volatile and constantly change places where congregate, either by seasonal, social, economic, or legal reasons (Leon et al., 2015; Tourangeau, Edwards, Johnson, Wolter, & Bates, 2014). If the study takes place over several months, it is also important to keep the database up to date, ensuring that venues and VDTs are the correct ones, eliminate the venues that have closed and ensuring the inclusion of venues that have opened in the meantime, otherwise will be another source of bias (Raymond et al., 2010; Semaan, 2010). Some low attendance or inaccessible VDTs excluded from the sampling frame might also increase selection bias (Marpsat & Razafindratsima, 2010). Another limitation concerns the generalisation of the results; it can only be inferred to key-population subgroups who frequent venues and not to the entire key-population, and this might be relevant because there are behavioural differences between subgroup members who do not visit venues and those who visit (de Sousa Mascena Veras et al., 2015).

TLS is an adequate method to survey disperse populations (Kalton, 2014), is one of the most used methods to conduct surveys and is also a recruitment technique recommended by some major organizations to collect data from key populations (UNAIDS, 2011).

The Sialon II, was a project conducted across 13 European countries with the objective of carry out and promote combined and targeted HIV/STI prevention for MSM. During the years of 2013-2014 the Sialon II project used the TLS method to conduct surveys in nine cities and countries: Belgium (Brussels), Bulgaria (Sofia), Germany (Hamburg), Poland (Warsaw), Portugal (Lisbon), Slovenia (Ljubljana), Spain (Barcelona), Sweden (Stockholm), and the UK (Brighton) (Gios et al., 2016).

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4 Relevance of the theme

International Health has a multidisciplinary and interdisciplinary approach aiming to improve health and well being of the most disadvantaged and vulnerable. Obtaining accurate HIV prevalence estimates of the most vulnerable groups is not an easy task. Identify members of these groups and select the best methods to sample them remains a challenge.

Aware of these handicaps and of the vital importance for international health to get accurate estimates for hard-to-reach populations, this thesis intends to contribute to improve the accuracy of HIV data through the application of a calibration method to the estimates obtained when TLS method is used to survey those populations.

This contribute will be based on a literature review of sampling methods used to reach hard populations, with the purpose of identifying the methods most used and its application contexts.

This contribute will also be based on the design of a calibration method to be applied to HIV estimates obtained when one of the methods most used to survey hard-to-reach populations is selected.

Finally, the contribute will be based on the application of the calibration method to two HIV prevalence studies from different countries, to confirm its performance.

5 Objectives

The general objective of this thesis is to assess the (empirical) validation of a new calibration method, called CARES, that improves the accuracy of HIV prevalence estimates obtained when time-location sampling method is used to survey hard-to reach-populations

The specific objectives are:

1) Identify all sampling methods used in International Health to survey Hard-to-Reach Populations of Men who Have Sex with Men and Sex Workers
2) Analyse conditions under which the sampling methods are applied

Study I: Hard-to-reach populations of men who have sex with men and sex workers: a systematic review on sampling methods

3) Develop a method that can be applied to time-location sampling samples to improve the accuracy of HIV estimates
4) Compare precision and accuracy of HIV estimates obtained using the method CARES with HIV estimates obtained using the time-location sampling method, through a simulation based on a Spanish dataset of men who have sex with men

Study II: Improving underestimation of HIV prevalence in surveys using time-location sampling

5) Confirm CARES performance by applying the method on other population, through a simulation based on a Portuguese survey of men who have sex with men

6) Compare CARES results on Portuguese and Spanish datasets and assess its performance

Study III: Calibration of HIV prevalence estimates in most-at-risk populations: results from a two countries simulation study

This thesis is therefore based on three papers, each with the respective background, methods, results, discussion and conclusion sections.

Part II – RESULTS

RESULTS

2.1 Hard-to-reach populations of men who have sex with men and sex workers: a systematic review on sampling methods

2.1.1 Abstract

Background: In public health, Hard to Reach Populations are often recruited by non-probabilistic sampling methods that produce biased results. In order to overcome this, several sampling methods have been improved and developed in the last years. The aim of this systematic review was identify all current methods used to survey most-at-risk populations of Men who have Sex with Men and Sex Workers. The review also aimed to assess if there were any relation between the study populations and the sampling methods used to recruit them. Lastly, we wanted to assess if the number of publications originated in MLHD countries had been increasing in the last years. ***Methods:*** A systematic review was conducted using electronic databases and a total of 268 published studies were included in the analysis. ***Results:*** In this review eleven recruitment methods were identified. Semi-probabilistic methods were used most commonly to survey Men who have Sex with Men and use of the Internet was the method that gathered more respondents We found that Female Sex Workers were more frequently recruited through non-probabilistic methods than Men who have Sex with Men (odds=2.2; $p<0.05$; CI [1.1-4.2]). In the last six years, the number of studies based in Middle and Low Human Development countries increased more than the number of studies based in Very High and High Human Development countries (odds=2.5; $p<0.05$; CI [1.3-4.9]). ***Conclusions***: This systematic literature review identified eleven methods used to sample Men who have Sex with Men and Female Sex Workers. There is an association between the type of sampling method and the population being studied. The number of studies based in Middle and Low Human Development countries has increased in the last six years of this study.

Key words: Hard to Reach Populations, Men who have Sex with Men, Sex Workers, sampling methods

2.1.2 Background

In public health, Hard to Reach Populations (HRP), hidden populations [1] or most-at-risk populations [2] are mainly associated with illegal or stigmatizing behaviours such as Sex Workers (SW), Injection Drug Users (IDU), Men who have Sex with Men (MSM) or Homeless People [3,4]. These groups are usually seen as key populations to be targeted as they have an important role on the spread of communicable diseases like HIV or Tuberculosis [5,6,7]. Thus, understanding how infectious epidemics affect them is crucial for the development of targeted and successful public health interventions. Ideally a representative sample of the study population should be selected and their socio-demographic characteristics and risk behaviours identified. However, most HRP do not have a sampling frame because their members are 'hidden', hence one cannot count how many they are [8,9]. On the other hand population-based surveys need to be very large to include enough 'hidden' members to get precise estimates, which is a limiting factor due mainly to the high costs. Therefore, studying HRP presents several difficulties and challenges: a) It is extremely difficult to use probability-sampling strategies to choose members to be included in the sample, and consequently non-probabilistic sampling methods are mainly used. The great disadvantage of these methods is that since they do not select individuals randomly the chosen elements may not be representative of the population to which they belong [10]; b) Each HRP has its own behavioural characteristics and deciding on which methods are the most adequate to use in each population is not straightforward [2]; c) In spite of the financial support most Middle and Low Human Development (MLHD) countries have been receiving for infectious diseases control [11], they still receive inadequate funding to reduce the vulnerability of HRP [12]. Consequently, the need persists for documenting trends on the HIV epidemics for these key populations in these regions [13,14].

Although several studies have been done in reviewing sampling methods [10,15,16,17,18], we did not find any systematic literature review.

The aim of this systematic review was identify all current methods used to survey most-at-risk populations of MSM and SW. The review also aimed to assess if there was any relation between the study populations and the sampling methods used to recruit them (that is to find if there is statistical significance between study populations and sampling methods). Lastly, we wanted to assess if the number of publications originated in MLHD countries had been increasing in the last years.

Data definitions

Populations

In public health, MSM is a term used to define men who engage in sex with other men irrespective of their sexual and gender identities. Commonly, this definition includes men who identify as gay, homosexual, bisexual, heterosexual and transgendered [19].

A transgender person (TG) is someone who has a gender identity different from his/her sex at birth. Transgender people may be male to female (female appearance) or female to male (male appearance) [20]. In this systematic review, the term "transgender" refers to the former definition because we did not find any study related to the latter.

Sex worker (SW) is defined as a person who receives money or goods in exchange for sexual services, and encompasses male (MSW), female (FSW) or transgender (TSW) people [21].

The term MSM is widely used in the literature not only to mean men who engage in sex with other men but also, men sex workers (MSW) and transgender people (sex workers or not) [22]. In order to be consistent with the current literature and for the purpose of analysis, retrieved publications were divided into subgroups consisting of Female Sex Workers and Men who have Sex with Men. Included in this last category are studies of Male Sex Workers, Transgendered persons, Transgender Sex Workers and Men who have Sex with Men.

Sampling Methods

We call Recruitment Methods the techniques applied to select a sample of elements from a target population. In this systematic literature review we identified eleven recruitment methods, which we grouped into three categories. More information about the retrieved methods can be seen in annex [S4].

The first category includes *non-probabilistic sampling methods* where the sampled elements are chosen arbitrarily or casually. In these methods it is not possible to estimate the probability of each element being included in the sample and consequently there is no way of making inferences to the population [3]. Methods that encompass the non-probabilistic category are Convenience, Purposive, Snowball and Targeted sampling.

The second category includes *probabilistic sampling methods*. These *methods* include those where every element in the population has a known probability of being included in the sample; the concept of p*robability sampling* means that a sample has been drawn in a probabilistic way [23] and consequently reliable estimates are produced and inferences can be made to the study population. Random Digit Dialling (RDD), Cluster sampling, Multi-stage sampling and Stratified Probability Sampling (SPS) are included in this probabilistic category.

The third category is the *semi-probabilistic*; this category includes methods that we believe do not fall in either of the other two because, from a *theoretical* point of view it is possible to determine the probability of each element being included in the sample, however, in practice, probabilities cannot be calculated [15,24] and therefore these methods do not allow making *reliable* inferences to the (unknown) population. Internet Sampling, Respondent Driven Sampling (RDS) and Time Location Sampling (TLS) are included in this category.

Countries

Countries where the studies were conducted were first classified into 8 regions UNAIDS [25]. Later, for the purpose of the analysis, these countries were classified in accordance with the development level, as defined by UNDP [26]. UNPD classifies countries in four levels of human development: Very High Human Development (VHHD), High Human Development (HHD), Medium Human Development (MHD) and Low Human Development (LHD). For our analysis we grouped the first two categories into one and named it as “Very High and High Human Development (VHHHD)” and grouped the last two categories into another one and named it “Medium and Low Human Development (MLHD)”.

2.1.3 Methods

The review was reported according to PRISMA recommendations [27].

Study selection

Electronic literature searches were conducted from 1st January 2003 to 31st December 2013 in the following databases: EBSCO, Gale, NLM (PubMed Central, PMC, Medline), Oxford University Press, ExLibris, Web of Knowledge, Elsevier, SpringerLink, Taylor & Francis Online, PLoS and SAGE. The search engine B_ON– Online Knowledge Library* was used to conduct database searches. The last search was run on June 16th, 2014. Studies were excluded if they did not include study participants (e.g., studies that make theoretical assumptions only), were systematic or non-systematic reviews, letters, editorials or commentaries. We also excluded clinical trials due to the very specific methods used to recruit participants, publications that did not mention the recruitment method and all publications that required additional payment for access.

In this first systematic literature review of methods used for sampling HRP, inclusion criteria were broad to cover as many publications as possible. The following list of key words was drawn up and were used as search terms: Men who have Sex with Men; Sex Work, Sex Workers, recruit, recruited, participants, enrol, enrolled, sample, sampling. The search terms Men who have Sex with Men, Sex Work and Sex Workers were included in the publication title, and in the abstract the remaining terms were included. In this manner, papers were selected if they mentioned at least one of the two above mentioned HRP and not less than two of the remaining search terms in the abstract according to the following two steps:

The first step was using the B-On database (capital letters indicate the search engine option): GLOBAL SEARCH; TITLE (EXACT): {sex work} OR {sex workers} OR {men who have sex with men}; ANY (CONTAINS): [{recruit} OR {recruited} OR {participants}] AND [{enrol} OR {enrolled} OR {participants}] AND [{sample} OR {sampling}]; TYPE OF MATERIAL: all publications; LANGUAGE: English; START DATE: 01/01/2003; END DATE: 31/12/2013. This search yielded 1707 publications.

The second step was used to narrow the initial search because the B-On search engine does not have the option to search in the abstract only. Therefore, retrieved publications were exported to the Reference Manager 12© software and the same key-words were applied to the abstracts (capital letters indicate the search engine option): TITLE: [{sex work} OR {sex workers} OR {men who have sex with men}] AND ABSTRACT: [{recruit} OR {recruited} OR {participants}] AND [{enrol} OR {enrolled} OR {participants}] AND [{sample} OR {sampling}]

This search identified 602 publications.

After duplicates had been deleted, titles and abstracts of all records were screened and exclusion and inclusion criteria were applied. Selected studies could use any design (other than randomized trial), must have referred to MSM and SW populations and have been public health related. Studies must have been written in English and be published between the beginnings of 2003 to the end of 2013. A second level of screening was applied to the full text for those studies that did not mention the sampling method in the title and/or abstract.

We only included studies that explicitly mentioned the recruitment method. The same reasoning was applied to those studies that used more than one recruitment method but only identified one of them, for instance; only the identified method was included in the review. One reviewer initially applied the exclusion and inclusion criteria in the first and second level of screening and then the second and third reviewer independently screened any studies that the first reviewer excluded to ensure that no relevant studies were accidentally left out of the review. The second and third reviewer also screened at random 20% of the studies that were included in the first and second level of screening to make sure the decision of inclusion was the correct one. Disagreements were resolved through discussion among all authors.

After selecting studies to be included in the systematic review, data about year of publication, country or region where the study took place, study population, sample size, and recruitment method were extracted to a spreadsheet by the first reviewer and then the second and third reviewer checked the extracted data for all studies. Disagreements were resolved through discussion among the authors.

Our study includes only descriptive data and therefore no assessment of risk of bias was done in individual studies or across studies.

Statistical methods

Statistical software SPSS® 22 for Mac OS was used to run the analysis. Descriptive statistics were used to analyse data. Odds ratio (OR) were calculated through logistic regression tool using the Wald Chi-square test and 95% confidence intervals (CI) were measured in order to assess if there was any association between studied populations and sampling methods and to appraise if the number of publications was associated with the regions where studies were carried out. P-values less than 0.05 were considered statistically significant.

2.1.4 Results

Study selection

Figure 1 presents the study selection process according to PRISMA 2009 flow diagram [27]. Our search identified 602 records of which 152 were removed as being duplicated. After screening titles and abstracts, 26 records were excluded for the reasons stated in the flow diagram and the recruitment methods were identified in 205 publications. During the process of screening the full text of the remaining 219 publications, another 156 articles were excluded and 63 publications were accessed. In the end, this review included 268 published articles for the analysis.

Study characteristics and findings

Recruitment methods

Table 1 presents the eleven sampling methods identified in this review. Respondent Driven Sampling (RDS) method was identified in 28.7% (n=77) of publications, and Time Location Sampling (TLS) was mentioned in 15.7% (n=42). Snowball sampling was used as a recruitment method in 13.4% (n=36) and Convenience and Internet methods were identified in 12.3% (n=33) of the publications. Targeted sampling and

Purposive sampling were mentioned in 5.6% (n=15) and 2.2% (n=6) of retrieved publications, respectively. Multi-Stage sampling, Cluster sampling and RDD were identified in 1.5% (n=4), 1.1% (n=3) and 0.7% (n=2) of the studies, respectively. In the remaining 6.3% of studies two or more methods were identified in each study and Stratified Sampling was used in one of these studies.

Internet was the sampling method that gathered more respondents with a total of 225320 participants in thirty-three selected studies. RDS gathered 82004 respondents in seventy-seven studies and 55193 respondents participated in the forty-two studies that used TLS method. Altogether, these three sampling methods recruited more than 84% of all respondents identified in the publications.

The identified recruitment methods were categorized into one of three categories (table 2): non-probabilistic (n=106), *semi-probabilistic* (n=170) and probabilistic (n=11). Methods included in the *semi-probabilistic category,* Internet, TLS and RDS, were applied in the most retrieved studies (59%) and were also those who recruited more respondents (more than 80%).

Populations and Regions

Table 3 presents all studies by study populations and category of method [see also table S1]. About 77% (n=205) of retrieved studies referred to MSM population, FSW were subject in about 18% (n=48), MSW were included in 1.5% (n=4) and TSW were identified in 0.4% (n=1) publications. The remaining 3.7% of studies related to more than one (sub) population: four studies were related to MSM/TG, three studies presented information about MSM/MSW/TG, and FSW/MSM, MSM/MSW and MSM/MSW/TSW were referred in one study each. About 83% (n=49) of Sex Work publications were Female Sex Work related.

Our search found that FSW are more frequently recruited by non-probabilistic methods than MSM (odds=2.2; $p<0.05$; CI [1.1-4.2]). Also, we found that the *Semi-probabilistic* methods were mainly used in studies of Men who have Sex with Men.

We identified 53 countries and regions [table S2] as being the origin of the identified studies. Countries were classified into 8 regions [table 4]. More than 70% of the studies were published in the regions of North America (40.7%, n=107) and Asia and Pacific (30%, n=81). North Africa and Middle East Region was the region that published fewer studies (0.8%, n=2).

Although most of the retrieved publications belong to the VHHHD regions (58%), our review found that, in the last six years, the number of studies published based in MLHD countries increased more than those based in VHHHD countries (odds=2.541; $p<0.05$; CI [1.327-4.866]) (table 5).

2.1.5 Discussion

This is to our knowledge the first systematic literature review of methods used to sample most-at-risk populations of FSW and MSM. We identified 268 published articles from 53 countries or regions and 11 recruitment methods. Over 427000 participants were surveyed in these 268 studies. Sampling methods we classified as semi-probabilistic (internet, TLS and RDS) were used in 59% of the retrieved studies. These results are consistent with prior studies finding that Web-based surveys, TLS and RDS methods have been used more extensively in health research in the past years [28,29,30]. The increase in the use of semi-probabilistic methods might be associated with the 2005 proposal of United Nations General Assembly Special Session[§] (UNGASS) that proposed a new set of indicators according to the level of epidemics in countries: generalized or concentrated [31]. Probabilistic methods were applied in less than 5% of the studies, which may be because implementing a probabilistic approach in most-at-risk populations is expensive, inconvenient or impossible [2].

Our systematic literature review found that FSW were more frequently recruited by non-probabilistic methods than were MSM, who were more often recruited with semi-probabilistic methods. The WHO suggests that the sampling strategies used to collect data on MSM should be RDS, TLS or cluster sampling while to collect data on FSW, TLS or convenience sampling should be used [2]. Our results are thus consistent with WHO suggestions and also in accordance with other studies that found FSW being mainly recruited by non-probabilistic methods [32] and MSM being primarily recruited by RDS, Internet and TLS [33,34] methods. *Semi-probabilistic* methods, namely RDS and Internet, require that target populations form some kind of social network [35].

Some studies reported that FSW usually have smaller peer network groups than other high-risk groups [36,37] and they also have few friends/close friends among recruiters [36], which might explain why this population is mainly recruited by non-probabilistic methods.

The aid to MLHD countries on STD control has increased about 75% between 2008-2013 when compared to the period of 2003-2007 [11]. This additional aid might be an explanation for the increasing number of publications once the number of published articles can be seen as an indicator of productivity [38].

Retrieved publications that employed *semi-probabilistic* methods gathered the greatest number of respondents. These methods have the advantage of reaching the most “hidden” individuals among “hidden” populations [32], which is a gain not only when compared to non-probabilistic methods but also when compared with probabilistic ones that always demand for a sampling frame and can miss many hidden individuals [1].

Providing unbiased estimates of prevalence for HIV surveillance is crucial for effective public health interventions [2]. However, reliable estimates cannot be produced without an appropriate sampling approach, which depends on several factors including the local context, availability of resources and the target population. Thus, the same method is not necessarily the best for all situations, populations and countries [34].

In practice, researchers studying HRP behaviours have to choose between several sampling methods and there are no precise guidelines in the literature to choose one method over another. Moreover, in this field, systematic reviews are particular challenging because public health problems require us to draw on complex sets of quantitative evidence [39]. This study provides researchers working in this area with a systematic evaluation of the sampling methods used by other researchers, and may be especially useful for readers and other investigators who consider new research projects that address hard to reach populations.

Limitations

This study has several limitations. One limitation is the definition of inclusion and exclusion criteria; searching for published studies only might have left behind many studies. Additionally, searching just for English written studies also leaves out studies published in other languages, which might be relevant for our purpose. Having the HRP name in the publication title may have excluded some potentially eligible studies from this review. Another limitation concerns the identification of populations and sub populations: we searched for MSM term in the title only which might have left out of this review several (sub)populations such as transgender or bisexual persons. Lastly, several publications were identified using the same database, hence the same recruitment strategy, which might have led to biased results.

2.1.6 Conclusion

This systematic literature review found that eleven methods had been used to sample MSM and FSW. These eleven methods were categorized in three categories. The semi-probabilistic category was the most commonly used method to survey MSM and the Internet was the method that gathered more respondents. Female Sex Workers were mainly recruited by non-probabilistic methods. Most published studies were originated in the regions of North America and Asia-Pacific. While most of the retrieved publications belong to the VHHHD regions, in the last six years, the number of studies published based in MLHD countries has been increasing.

List of abbreviations

Female Sex Worker (FSW), Hard to Reach Populations (HRP), Injection Drug Users (IDU), Joint United Nations Programme on HIV/AIDS (UNAIDS), Male Sex Worker (MSW), Men who have Sex with Men (MSM), Organisation for Economic Co-operation and Development (OECD), Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), Primary Sampling Units (PSUs), Random Digit Dialling (RDD), Respondent Driven Sampling (RDS), Second Sampling Units (SSUs), Sex Workers (SW), Sexually Transmitted Diseases (STD), Time Location Sampling (TLS), Time Space Sampling (TSS), Transgender Person (TG), Transgender Sex Worker (TSW), United Nations General Assembly Special Session (UNGASS).

Endnotes

*http://www.b-on.pt/index.php?lang=en

§ The UNGASS is a meeting of the United Nations member states to assess and discuss global topics such as health, gender, or drugs priorities. In 2005 a declaration of commitment of member states was produced as a result from the UNGASS meeting in 2001. This declaration of commitment is the result of the global consensus that member-states reached in order to achieve the Millennium Development Goals of halting and reverse the HIV epidemics. Several key indicators were then proposed to measure the effectiveness of the response of each country in fighting HIV (http://data.unaids.org/publications/irc-pub06/jc1126-constrcoreindic-ungass_en.pdf)

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2.1.8 Annexes

Tables

TABLE 1: DESCRIPTIVE STATISTICS OF THE RETRIEVED PUBLICATIONS

Recruitment methods	Number of studies	%	Total sample sizes	Mean	Median	Minimum	Maximum	Study reference[#]
RDS	77	28.7	82004	1065	496	50	18960	[1–39][40–77]
TLS	42	15.7	55193	1346.2	526	200	16270	[78–119]
Convenience	33	12.3	18698	584.3	305.5	36	2569	[120–152]
Snowball	36	13.4	18148	504.1	331.5	20	3314	[153–188]
Internet	33	12.3	225320	6827.9	522	32	144177	[189–221]
Targeted	15	5.6	6197	413.1	485	22	806	[222–236]
Purposive	6	2.2	195	32.5	26.5	17	58	[237–242]
Multi-stage probability sample	4	1.5	3175	793.8	216	121	2622	[243–246]
Cluster	3	1.1	1901	633.7	504	324	1073	[247–249]
Convenience, Snowball	3	1.1	687	229	253	161	273	[250–252]
TLS, Internet	2	0.7	1167	583.5	583.5	566	601	[253,254]
RDD	2	0.7	2659	1329.5	1329.5	879	1780	[255,256]
Convenience, Internet	2	0.7	2291	1145.5	1145.5	770	1521	[257,258]
Convenience, RDD	1	0.4	218					[259]
Convenience, RDS	1	0.4	624					[260]
Internet, Snowball	1	0.4	1692					[261]
RDS, TLS	2	0.7	1853	926.5	926.5	737	1116	[262,263]

Stratified probability sampling, Internet	1	0.4	2182	[264]
Targeted, Snowball	1	0.4	48	[265]
Convenience, Snowball, Internet	1	0.4	103	[266]
Snowball, TLS, RDS	1	0.4	1407	[267]
RDS, Internet	1	0.4	2147	[268]
Total	**268**	**100**	**427909**	

#References can be found in text S3

TABLE 2: RECRUITMENT METHODS BY CATEGORIES

Recruitment methods	Form	Category
Convenience	Venue-based	Non-Probabilistic
Purposive	Link-tracing	Non-Probabilistic
Snowball	Link-tracing	Non-Probabilistic
Targeted	Link-tracing	Non-Probabilistic
Internet	Venue-based	Semi-Probabilistic
RDS	Link-tracing	Semi-Probabilistic
TLS	Venue-based	Semi-Probabilistic
Cluster	Probabilistic	Probabilistic
Multi-stage probability sample	Probabilistic	Probabilistic

RDD	Probabilistic	Probabilistic
Stratified probability sampling	Probabilistic	Probabilistic

TABLE 3: STUDY POPULATIONS BY CATEGORIES

Populations	Non-Probabilistic N (%)	Semi-Probabilistic N (%)	Probabilistic N (%)	Mixed[##] N (%)	Total N (%)	References[#]
MSM	64 (23.9)	128 (47.8)	6 (2.2)	7 (2.6)	205 (76.5)	[1–5,7,78–80,82–84,120–126,153,155,156,158–160,189–194,222,223,237,255][11–13,15–18,20,85,87–89,127,128,131–135,162–164,167,168,196–200,224–226,228,243,253,254,256,260,264,267][22–25,29–31,35–40,90–104,137,138,169–171,175,176,178,201–204,206–209,229,230,244,250,265,266][42–50,52,53,56–58,60–62,105–107,109,111–113,139,143–147,149,150,179–181,183,184,210–218,245,246,259,261][64,67–76,115,116,118,119,152,185–188,219–221,236,242,258,263,268]
FSW	24 (9.0)	21 (7.8)	3 (1.1)	0	48 (17.9)	[8–10,14,19,21,26–28,33,34,41,51,54,55,59,63,66,77,117,129,136,140,142,151,157,161,165–167,172–174,177,182,227,231–235,239,241,247–249,251,262]
MSW	2 (0.7)	2 (0.7)	0	0	4 (1.5)	[108,154,205,252]
TSW	1 (0.4)	0	0	0	1 (0.4)	[148]
MSM/TG	3 (1.1)	1 (0.4)	0	0	3 (1.5)	[6,141,238,240]
FSW/MSM	0	1 (0.4)	0	0	1 (0.4)	[32]
MSM/MSW	0	1 (0.4)	0	0	1 (0.4)	[114]
MSM/MSW/TG	0	3 (1.1)	0	0	3 (1.1)	[81,86,110]
MSM/MSW/TSW	0	0	0	1 (0.4)	1 (0.4)	[257]
Total	**94 (35.1)**	**157 (58.6)**	**9 (3.4)**	**8 (3.0)**	**268 (100.0)**	

##Publications that mentioned at least two categories of recruitment methods

#References can be found in text S3

TABLE 4: PUBLICATIONS BY REGION

Regions	Countries (N)	Publications (%)
Asia and Pacific	12	81(30.8)
East and Southern Africa	7	20 (7.6)
Eastern Europe and Central Asia	7	11 (4.2)
Latin America	7	21(8.0)
North Africa and Middle East	2	2 (0.8)
North America	2	107 (40.7)
West and Central Africa	4	8 (3.0)
West and Central Europe	7	13 (4.9)
Total	**48**	**263**

Five publications were excluded for belonging to more than one region

TABLE 5: POPULATIONS AND YEARS OF PUBLICATION BY REGION'S DEVELOPMENT LEVEL

Years	MLHD countries (%)	VHHHD countries (%)	Total
2003-2007	15 (29.4)	36 (70.6)	51
2008-2013	95 (44.8)	117 (55.2)	212

Five publications were excluded for belonging to more than one region

Additional Files

Table S1 Recruitment methods by population type

Method	MSM (%)	FSW (%)	MSW (%)	TSW (%)	MSM/TG (%)	FSW/MSM (%)	MSM/MSW (%)	MSM/MSW/TG (%)	MSM/MSW/TSW (%)	Total (%)
RDS	56 (20.9)	19 (7.1)	0	0	1 (0.4)	1 (0.4)	0	0	0	77 (28.7)
TLS	36 (13.4)	1 (0.4)	1 (0.4)	0	0	0	1 (0.4)	3 (1.1)	0	42 (15.7)
Convenience	26 (9.7)	5 (1.9)	0	1 (0.4)	1 (0.4)	0	0	0	0	33 (12.3)
Snowball	25 (9.3)	10 (3.7)	1 (0.4)	0	0	0	0	0	0	36 (13.4)
Internet	32 (11.9)	0	1 (0.4)	0	0	0	0	0	0	33 (12.3)
Targeted	9 (3.4)	6 (2.2)	0	0	0	0	0	0	0	15 (5.6)
Purposive	2 (0.7)	2 (0.7)	0	0	2 (0.7)	0	0	0	0	6 (2.2)
Multi-stage probability sample	4 (1.5)	0	0	0	0	0	0	0	0	4 (1.5)
Cluster	0	3 (1.1)	0	0	0	0	0	0	0	3 (1.1)
Convenience, Snowball	1 (0.4)	1 (0.4)	1 (0.4)	0	0	0	0	0	0	3 (1.1)

TLS, Internet	2 (0.7)	0	0	0	0	0	0	0	0	2 (0.7)
RDD	2 (0.7)	0	0	0	0	0	0	0	0	2 (0.7)
Convenience, Internet	1 (0.4)	0	0	0	0	0	0	0	1 (0.4)	2 (0.7)
Convenience, RDD	1 (0.4)	0	0	0	0	0	0	0	0	1 (0.4)
Convenience, RDS	1 (0.4)	0	0	0	0	0	0	0	0	1 (0.4)
Internet, Snowball	1 (0.4)	0	0	0	0	0	0	0	0	1 (0.4)
RDS, TLS	1 (0.4)	1 (0.4)	0	0	0	0	0	0	0	2 (0.7)
Stratified probability sampling, Internet	1 (0.4)	0	0	0	0	0	0	0	0	1 (0.4)
Targeted, Snowball	1 (0.4)	0	0	0	0	0	0	0	0	1 (0.4)
Convenience, Snowball, Internet	1 (0.4)	0	0	0	0	0	0	0	0	1 (0.4)
Snowball, TLS, RDS	1 (0.4)	0	0	0	0	0	0	0	0	1 (0.4)
RDS, Internet	1 (0.4)	0	0	0	0	0	0	0	0	1 (0.4)
Total	**205 (76.5)**	**48 (17.9)**	**4 (1.5)**	**1 (0.4)**	**4 (1.5)**	**1 (0.4)**	**1 (0.4)**	**3 (1.1)**	**1 (0.4)**	**268**

Table S2 Country/region of origin by population type

Country	MSM	FSW	MSW	TSW	MSM/TG	FSW/MSM	MSM/MSW	MSM/MSW/TG	MSM/MSW/TSW	Total (%)	References
Afghanistan	0	1	0	0	0	0	0	0	0	1 (0.4)	[151]
Argentina	4	0	0	0	0	0	0	0	0	4 (1.5)	[7,40,69,183]
Asia	1	0	0	0	0	0	0	0	0	1 (0.4)	[203]
Australia	1	1	0	0	0	0	0	0	0	2 (0.8)	[140,207]
Benin, Guinea, Senegal	0	1	0	0	0	0	0	0	0	1 (0.4)	[129]
Brasil	5	3	0	0	1	0	0	0	0	9 (3.4)	[2,6,8,9,39,54,72,160,267]
Cameroon	2	0	0	0	0	0	0	0	0	2 (0.8)	[70,171]
Canada	2	0	0	0	0	0	0	0	0	2 (0.8)	[100,145]
China	31	14	1	0	0	0	1	0	0	47 (17.5)	[12,15,26–31,58–62,75,76,103,114,130,132,147, 149,150,158,159,161,162,165–170,172,177,179,184,186,188, 212,218,241,247,249,252,260–262]
Croatia	4	0	0	0	0	0	0	0	0	4 (1.5)	[4,5,25,180]
Croatia, Montenegro	0	1	0	0	0	0	0	0	0	1 (0.4)	[251]
El Salvador	1	0	0	0	0	0	0	0	0	1 (0.4)	[64]
Estonia	1	1	0	0	0	0	0	0	0	2 (0.8)	[55,146]
Europe	2	0	0	0	0	0	0	0	0	2 (0.8)	[126,219]
Gambia	1	0	0	0	0	0	0	0	0	1 (0.4)	[187]

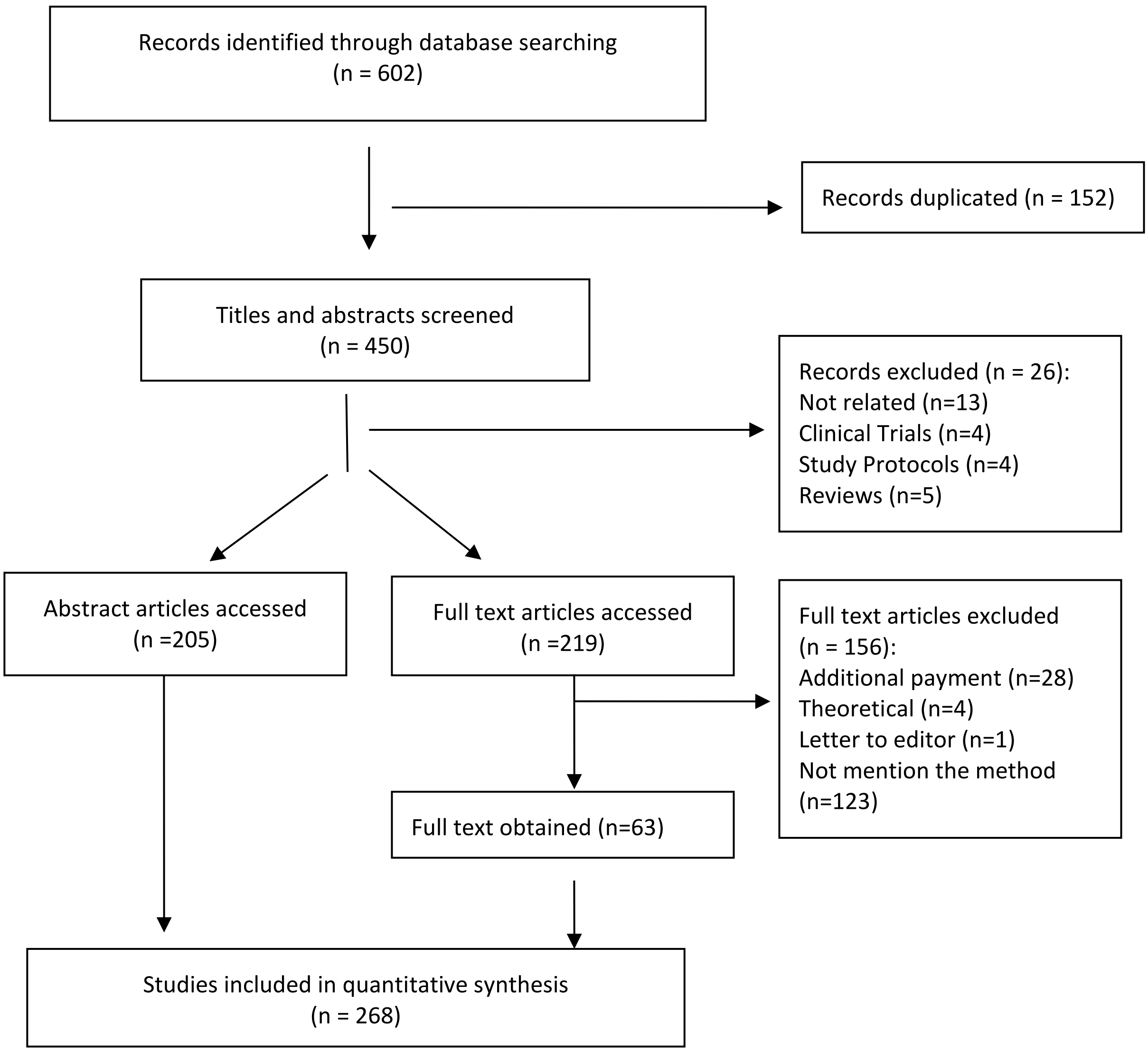
Germany	1	0	0	0	0	0	0	0	0	1 (0.4)	[204]
Guatemala	1	0	0	0	1	0	0	0	0	2 (0.8)	[238,263]
Honduras	0	1	0	0	0	0	0	0	0	1 (0.4)	[21]
India	5	8	0	0	1	0	0	0	0	14 (5.2)	[10,14,33,34,41,51,53,63,102, 133,139,141,181,239]
Indonesia	0	0	0	0	0	0	0	0	1	1 (0.4)	[257]
Israel	1	1	0	0	0	0	0	0	0	2 (0.8)	[136,138]
Japan	1	0	0	0	0	0	0	0	0	1 (0.4)	[197]
Kazakhstan	1	0	0	0	0	0	0	0	0	1 (0.4)	[3]
Kenya	1	3	0	0	0	0	0	0	0	4 (1.5)	[157,173–175]
Lao	1	0	0	0	0	0	0	0	0	1 (0.4)	[105]
Lebanon	0	0	0	0	0	1	0	0	0	1 (0.4)	[32]
Malawi	1	0	0	0	0	0	0	0	0	1 (0.4)	[73]
Malawi, Namibia, Botswana	1	0	0	0	0	0	0	0	0	1 (0.4)	[155]
Malaysia	1	0	0	0	0	0	0	0	0	1 (0.4)	[90]
Mexico	1	1	0	0	0	0	0	0	0	2 (0.8)	[121,142]
Moldova	0	1	0	0	0	0	0	0	0	1 (0.4)	[77]
Mongolia	1	0	0	0	0	0	0	0	0	1 (0.4)	[74]
Netherlands	3	0	0	0	0	0	0	0	0	3 (1.1)	[189,217,222]
Nigeria	4	0	0	0	0	0	0	0	0	4 (1.5)	[1,143,185,237]
Peru	2	0	0	0	0	0	0	0	0	2 (0.8)	[115,123]
Russia	0	0	1	0	0	0	0	0	0	1 (0.4)	[154]
Russia, USA	1	0	0	0	0	0	0	0	0	1 (0.4)	[17]
Singapore	1	0	0	0	0	0	0	0	0	1 (0.4)	[268]

South Africa	9	1	0	0	0	0	0	0	0	10 (3.7)	[22,48,49,56,78,152,153,176,213,235]
South Korea	0	2	0	0	0	0	0	0	0	2 (0.8)	[117,248]
Spain	1	0	0	0	0	0	0	0	0	1 (0.4)	[128]
Swaziland	1	0	0	0	0	0	0	0	0	1 (0.4)	[71]
Sweden	1	0	0	0	0	0	0	0	0	1 (0.4)	[210]
Switzerland	1	0	0	0	0	0	0	0	0	1 (0.4)	[120]
Tanzania (Zanzibar)	1	0	0	0	0	0	0	0	0	1 (0.4)	[20]
Thailand	3	1	1	0	0	0	0	3	0	8 (3.0)	[66,81,86,97,98,108–110]
Uganda	2	0	0	0	0	0	0	0	0	2 (0.8)	[16,65]
UK	5	0	0	0	0	0	0	0	0	5 (1.9)	[11,144,195,258,264]
USA	95	5	1	1	1	0	0	0	0	103 (38.4)	[13,79,80,82–85,87,122,124,125,127,131,156,163,164,190–194,196,198,199,223–228,243,253–256][18,23,24,35–38,88,89,91–95,99,134,135,137,200–202,205,206,208,229,244,266] [42-47,50,52,57,67,68,101,104,106, 107,111,113,116,118,119,148,178,209,211,214,216,220,221,230–234,236,240,245,246,250,259,265]
USA, Puerto Rico	1	0	0	0	0	0	0	0	0	1 (0.4)	[96]
USA,	1	0	0	0	0	0	0	0	0	1 (0.4)	[215]

Canada											
Uzbekistan	0	1	0	0	0	0	0	0	0	1 (0.4)	[182]
Vietnam	1	1	0	0	0	0	0	0	0	2 (0.8)	[19,242]
Total	**205**	**48**	**4**	**1**	**4**	**1**	**1**	**3**	**1**	**268 (100)**	

Text S1 PRISMA flow diagram

Text S1: PRISMA Flow Diagram on Strategies Used for Sampling Hard to Reach Populations in Public Health



Text S2 PRISMA checklist

Text S2: PRISMA checklist

Section/topic	*#*	*Checklist item*	*Reported on page #*
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 3-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g.,	Page 6-7

Section/topic	*#*	*Checklist item*	*Reported on page #*
		years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 6-7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Page 7-8
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 4-6 ‘Data Definitions’
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication	Page 9

Section/topic	*#*	*Checklist item*	*Reported on page #*
		bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 9 and additional file: text S1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 9-11 and tables 1-5
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see Item 12).	NA
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot.	a)Table 1, table 3 and additional file: table S2 b)NA
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 9-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA

Section/topic	*#*	*Checklist item*	*Reported on page #*
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., health care providers, users, and policy makers).	Page 11-14
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias).	Page 16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	None

Text S3 List of retrieved publications analysed in the systematic literature review

1. Adebajo SB, Eluwa GI, Allman D, Myers T, Ahonsi BA: **Prevalence of internalized homophobia and HIV associated risks among men who have sex with men in Nigeria**. Afr J Reprod Health 2012, 16: 21-28.

2. Bermudez-Aza EH, Kerr LRFS, Kendall C, Pinho AA, de Mello MB, Mota RS, Guimaraes MDC, Alencar CS, de Brito AM, Dourado IC, da Batista SMB, Abreu F, de Oliveira LC, Moraes AD, Benzaken AS, Merchan-Hamann E, de Freitas GMB, McFarland W, Albuquerque E, Rutherford GW, Sabino E: **Antiretroviral Drug Resistance in a Respondent-Driven Sample of HIV-Infected Men Who Have Sex With Men in Brazil**. Jaids-Journal of Acquired Immune Deficiency Syndromes 2011, 57: S186-S192.

3. Berry M, Wirtz AL, Janayeva A, Ragoza V, Terlikbayeva A, Amirov B, Baral S, Beyrer C: **Risk Factors for HIV and Unprotected Anal Intercourse among Men Who Have Sex with Men (MSM) in Almaty, Kazakhstan**. Plos One 2012, 7:

4. Bozicevic I, Lepej SZ, Rode OD, Grgic I, Jankovic P, Dominkovic Z, Lukas D, Johnston LG, Begovac J: **Prevalence of HIV and sexually transmitted infections and patterns of recent HIV testing among men who have sex with men in Zagreb, Croatia**. Sexually Transmitted Infections 2012, 88: 539-544.

5. Bozicevic I, Rode OD, Lepej SZ, Johnston LG, Stulhofer A, Dominkovic Z, Bacak V, Lukas D, Begovac J: **Prevalence of Sexually Transmitted Infections Among Men Who Have Sex with Men in Zagreb, Croatia**. Aids and Behavior 2009, 13: 303-309.

6. Carballo-Dieguez A, Balan I, Dolezal C, Mello MB: **Recalled Sexual Experiences in Childhood with Older Partners: A Study of Brazilian Men Who Have Sex with Men and Male-to-Female Transgender Persons**. Archives of Sexual Behavior 2012, 41: 363-376.

7. Carballo-Dieguez A, Balan I, Marone R, Pando MA, Dolezal C, Barreda V, Leu CS, Avila MM: **Use of Respondent Driven Sampling (RDS) Generates a Very Diverse Sample of Men Who Have Sex with Men (MSM) in Buenos Aires, Argentina**. Plos One 2011, 6:

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Text S4: Sampling methods identified in the systematic review

Convenience sampling method is set of techniques in which respondents are selected by convenience due to their proximity, availability, accessibility or other way that researcher decides [1]. It is a fast and easy method to use however results seldom are representative of the population [2].

Purposive sampling is a technique in which researcher, with a particular purpose, chooses the respondents that participate in the study [1]. It is useful when researchers are looking for persons with a specific characteristic (sometimes rare) but, because it is a non-probability based sampling it does not allow to make inferences to the population.

Snowball sampling is a method of chain referral in which researchers contact members of the target population, already known to them, who are invited to participate in the survey and are in turn asked to refer their peers and/or help researchers to identify them [3]. The chain referral method allows researchers reach populations that are hard to sample using other methods, nevertheless individuals who have a large number of social connections are able to nominate peers that have the same characteristics as their own, and thus the sample may not be representative of the population [4].

Targeted sampling consists in doing an initial ethnographic assessment in order to identify subpopulations or subgroups with some specific attributes and then elaborate a plan to recruit members with those characteristics where the members of the population might be found [5]. This method offers an approach that can be useful to identify social and cultural characteristics of subgroups as well as their geographic distribution, but the sample will be biased towards those who gather in the selected location [5], is labour intensive and time consuming.

Random Digit Dialling (RDD) is a probabilistic method used for selecting respondents from a set of telephone numbers (usually landlines) [6]. It has the advantage of reaching a geographic dispersed sample and including those who live in rural areas, it is time and cost saving because interviewers do not have to physically go to study areas. Among limitations of RDD there is the possible lack of representativeness, as not all persons have a landline, and the high rate of unfruitful calls [7].

Multi-stage sampling is a probabilistic method that consists of more than two stages of sampling; the first stage identifies the primary sampling units (PSUs) (e.g. geographical areas), the second stage selects the units within the PSU (Second Sampling Units – SSUs)(e.g. hospitals within geographical areas), the third stage selects the units within the SSUs (e.g. hospital units), and so on [8].

Cluster sampling is a special case of a multi-stage sampling, where the study population is divided into groups (or clusters) and then a sample of those clusters is selected [8,9].

In stratified probability sampling method the population members are divided into homogeneous groups (called strata) such as regions or age categories for instance, and then a sample is selected (usually a simple random sampling or systematic sampling) within each strata [9].

When specifically talking about surveying HRP, all probabilistic methods, like those presented here have the great disadvantage of the cost because most hidden populations, like MSM and FSW, are a minority in the general population, thus collecting a probability sample would be too expensive [2].

Internet sampling technique consists in recruiting respondents through the internet, either through advertising or contact people directly (through chat rooms for instance); we categorized in this method all publications that mentioned that respondents were recruited through “internet”, “on-line” or via “web”, no matter if it was a web-based survey or if the internet was used only to advertise the research [10]. This method offers a mechanism through which a researcher can have access to people who share similar characteristics, interests or attitudes. It is time and cost saving however little may be known about the characteristics of the online community members; it may not be easy to define a sampling frame if the researcher does not have access to the number of members of a community or their email addresses. Non-response rates are also hard to identify for most online communities [11].

Time Location Sampling (TLS) also called Time Space Sampling (TSS) consists in identifying the venues and time periods where the study population congregates and then select a sample of sites to recruit members during a pre-defined time interval [12]. TLS is an efficient way to sample hidden populations that congregate in specific locations and is able to approximate probability sampling [13], however not all select places are easily accessible and others are not even contemplated due to safety reasons or to high costs [14]. Besides, populations that congregate at public venues may differ from the true population as some of them may only frequent private venues. This means that there might be an unknown potential bias in the estimates [14].

Respondent Driven Sampling (RDS) is chain referral technique in which researchers contact a predefined small number of population members called seeds and asks them to recruit their peers to participate in the survey. If the peers are eligible for the study they are invited to become seeds and to recruit other members. This technique gives incentives to peers and to seeds who recruits them [15]. RDS has the advantage of recruiting individuals that do not congregate in public venues, however it will not function if the study population are not socially networked [16]. A major difference from Snowball sampling is that RDS relies on elements of the target population to recruit their peers using a set of coded coupons that are redeemed. The coupon quota reduces biases associated with over representation of those who have large networks [4]. Also in order to reduce bias, during the survey, data on network structure are also collected and used to determine post-hoc sampling weights [17].

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2.2 Improving underestimation of HIV prevalence in surveys using time-location sampling

2.2.1 Abstract

We sought to find a method that improves HIV estimates obtained through time location sampling (TLS) used to recruit most-at-risk populations (MARPs). The calibration on residuals (CARES) method attributes weights to TLS sampled individuals depending on the percentile to which their logistic regression residues belong. Using a real country database, provided by EMIS-2010, with 9591 men who have sex with men (MSM) and an HIV prevalence of 12.1% we simulated three populations (termed "pseudo-populations") with different levels of HIV. From each pseudo-population 1000 TLS samples were drawn and the HIV prevalence estimated by the TLS method and by the CARES method were recorded and compared with the HIV prevalence of the 9591 men. Results showed that the CARES method improves estimates given by the TLS method by getting closer to the real HIV prevalence.

2.2.2 Background

Accurate estimates of HIV are essential to assess future healthcare needs, track the course of the epidemics, and evaluate the success of past intervention plans. However, data obtained for HIV prevalence tend to be underestimated[1,2] mainly because individuals who are HIV positive are less likely to participate in surveys[2] and those who do participate tend not to disclose their serostatus or their risk behaviors.[3,4] Studies based on self-reported data tend to underestimate HIV prevalence also because those unaware of their serological status and those who report being HIV negative may have seroconverted since their last negative test.[5–7]

Most-at-risk populations (MARP), also called key populations, are mainly associated with illegal or stigmatized behaviors such as sex workers (SW), men who have sex with men (MSM), or injection drug users (IDU).[8,9] Globally, SW are 13 times more likely to become HIV positive, IDU are 22 times more likely, and MSM are 28 times more likely than the rest of the adult population[9], and are therefore seen as significant populations contributing to the spread of HIV.[8,10] Studies targeting these populations face an additional difficulty of obtaining reliable estimates because there is no sampling frame[11] and it is therefore not possible to identify them or count how many they are.[10,12,13] For this reason, non-probabilistic and semi-probabilistic methods are the most used to survey those populations.[10,11]

Time-location-sampling (TLS), also called time-space sampling or venue-based sampling, is a widely used technique to sample MARPs which belongs to semi-probabilistic methods[14,15] and is an adaptation of multistage cluster sampling used to survey populations who frequent known locations. TLS consists of mapping the physical venues (bars, night-clubs, saunas, etc.), days and times (blocks of time are constructed depending on the number of hours a venue is open, e.g. every four hours is a block) when the target population congregates to build the sampling frame. The venues may be included in the sampling frame more than once at various times of the day or week. Combination of location and time is known as a sample event (also called cluster or primary sampling unit). The sample events are randomly selected and within each one participants meeting the eligibility criteria are casually or systematically selected to be interviewed. Each participant should be enrolled only once and the total number of attendees present in the venue should be identified to build the sampling weights.[14–16] The use of sampling weights and the frequency of venue attendance (FVA) are recommended to ensure the validity of TLS estimates and the representativeness of the samples.[16–19] Nevertheless, it seems that this practice is seldom applied.[19–21] The use of weights makes assumptions that are difficult to prove and to measure and therefore estimates might also be biased.[10,15,19] Consequently, the accuracy of estimates obtained through TLS is not consensual and is still subject to proposals for its improvement.[17,19,21]

In light of all these uncertainties we developed an approach that improves both weighted and non-weighted TLS results. In this paper we present a new method that improves the HIV prevalence estimated through TLS that uses a computationally intensive simulation. Using a database from a MSM survey, we simulated three populations with different levels of HIV prevalence, and from each one TLS samplings were drawn and a logistic regression model was run. This new method calibrates the TLS HIV prevalence estimates using the logistic regression residuals, and we call it CARES (Calibration on Residuals).

The goal of this study is to evaluate if the CARES method improves the TLS HIV estimated prevalence; i.e. to appraise the performance of the CARES method considering populations with distinct HIV prevalence levels and to attest the performance of CARES method considering different TLS sizes, with and without sampling weights.

2.2.3 Methods

Population

For the simulation we used the Spanish sub-sample (N=13111) of the European MSM Internet Survey (EMIS-2010) and limited our analysis to men reporting a previous HIV test. The purpose of EMIS was to develop a pan-European online survey with respect to prevention needs among European MSM.[22] International MSM dating-websites were contacted to send instant messages to their members to fill-in an online survey. Data were collected between June and August 2010, with a final dataset of 174,209 cases from 38 countries.[22] All data were self-reported. Details of this survey can be found elsewhere.[22–24]

Respondents who self-identified as men and who reported having ever received an HIV test result were selected as our study population. The study population had 9591 individuals of which 12.1% reported having been diagnosed with HIV. We use this as a proxy for HIV prevalence. Respondents who did not report the region were they were living were excluded. Regions with fewer than 150 respondents were left out because the TLS method tends to include in a survey physical venues where many participants congregate because the probability of selecting respondents is greater.[15] Additionally, about 25% of the study population was intentionally left out (randomly selected) because key populations and data obtained for HIV prevalence tend to be underestimated.[1,25] Therefore the population-base data used to simulate our study had 6185 individuals and an HIV prevalence of 11.74%.

Sampling

The question “which region do you live in?” included in the EMIS questionnaire was used to create the sample events (primary sampling unit or clusters). For simplicity we assumed only one time interval.[26] The population-base data were divided into 68 clusters ranging from 34 to 140 persons randomly allocated by cluster and considering that the number of persons by cluster should be proportional to the number of respondents in the respective region. The population-base was used to create three pseudo-populations with different levels of HIV prevalence. Pseudo-population 1 with an HIV prevalence of 9.87%, pseudo-population 2 with an HIV prevalence of 10.58%, and pseudo-population 3 with an HIV prevalence of 11.41%.

Simulations were run according to a two-stage sampling design as well established for MARPs.[15,16,26] From each pseudo-population samples were drawn using the TLS method in accordance with the following description: in the first stage, the sampling design probability proportional to size (PPS) was used to select 25% and 20% of the clusters in the first stage and in the second stage it was assumed that about 70% of the respondents from each selected cluster answered the questionnaire.[26] The number of persons in the venue at the time of the interview was also assumed to be known in order to construct the sampling weights.[26] Using pseudo-population 1, 1000 samples were drawn selecting 25% of the clusters and another 1000 samples were drawn selecting 20% of the clusters; the same procedure was done using pseudo-population 2 and pseudo-population 3. The lack of a standard measure can jeopardize the validation of any weighting procedure, and for this reason in this first attempt to provide a method that improves the TLS HIV estimates we assumed that all sampling units had equal probability of inclusion within the cluster. Weighted and unweighted prevalence of HIV infection was recorded as a mean of each 1000 samples drawn.

The method

The method calibrates the TLS HIV prevalence estimates using the logistic regression residuals. The logistic regression model is widely used in cases in which the outcome variable is binary or dichotomous and can be presented as:[27]

$$\boldsymbol{Y = \pi(x) + r} \quad \textbf{or} \quad \boldsymbol{Y = \frac{e^Z}{1+e^Z} + r} \qquad (1)$$

where

$\pi(x) = E(Y|x)$ represents the conditional mean of Y given x, $Z = \beta_0 + \beta_1 x$, represents the covariates, and r is the residual or error term and expresses an observation's deviation from the conditional mean.

Residuals are defined as the difference between the observed and fitted value and may assume one of two possible values:

$$\boldsymbol{r = 1 - \pi(x)} \text{ if } \boldsymbol{Y = 1} \quad \text{or} \quad \boldsymbol{r = -\pi(x)} \text{ if } \boldsymbol{Y = 0} \tag{2}$$

In logistic regression there are several possible ways to measure the difference between the observed and fitted values. We used the two most common types of measures. The first is called the Pearson residual, and is based on the idea of subtracting off the mean and dividing by the standard deviation:

$$\boldsymbol{r(y_i, \hat{\pi}_i) = \frac{(y_i - n_i\hat{\pi}_i)}{\sqrt{n_i\hat{\pi}_i(1-\hat{\pi}_i)}}} \tag{3}$$

where n_i subjects share the ith covariate pattern and y_i of them experience the event of interest. Combining the Pearson residuals produces the *Pearson chi-square statistic*: $X^2 = \sum r_i^2$.

The second type is the deviance residual:

$$\boldsymbol{d(y_i, \hat{\pi}_i) = \pm\sqrt{2\left[y_i ln\left(\frac{y_i}{n_i\hat{\pi}_i}\right) + (n_i - y_i) ln\left(\frac{(n_i - y_i)}{n_i(1-\hat{\pi}_i)}\right)\right]}} \tag{4}$$

where the sign is positive when $y_i \geq \hat{\pi}_i$ and negative otherwise. The summary statistic based on deviance residuals is the deviance: $D = \sum d_i^2$.

The proposed method (CARES) consists of assigning different weights to individuals according to the percentile their residuals belong to. Therefore the CARES weights are distributed as follows:

$$\boldsymbol{CARES = \begin{cases} a & if\ r < z \\ b & if\ r \geq z \end{cases}} \tag{5}$$

where a and b are the weights, r represents the residuals, and z is the percentile of the residuals.

Simulation

For each TLS sample drawn, a logistic regression model was run with HIV (diagnosed HIV positive /last HIV test negative) as the outcome variable and “age”(up to 24 years / 25-49 years / 50 years or more), “having a relationship with a steady partner”(yes/no), “had STI [Sexually Transmitted Infection] symptoms in the last 12 months”(yes/no), “age at first anal intercourse”(up to 15 years/16 or more), “had unprotected anal intercourse the last time had sex” (yes/no), “ever taken illicit drugs” (yes/no), and “education level”(low/mid/high) as covariates for HIV prevalence in MSM according to the literature. [28–30] The stepwise regression method and a significance level of the chi-square score of 0.1 were used for entering variables into the model. Pearson and Deviance logistic regression residuals were recorded as standardized values. The CARES method was tested with several weights applied according to different percentiles of residues in order to identify the best performance as defined below:

$$\boldsymbol{CARES_{12}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 5} \\ \mathbf{2} & \boldsymbol{if\ r \geq 5} \end{cases} \qquad \boldsymbol{CARES_{13}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 5} \\ \mathbf{3} & \boldsymbol{if\ r \geq 5} \end{cases}$$

$$\boldsymbol{CARES_{12}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 5.5} \\ \mathbf{2} & \boldsymbol{if\ r \geq 5.5} \end{cases} \qquad \boldsymbol{CARES_{13}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 5.5} \\ \mathbf{3} & \boldsymbol{if\ r \geq 5.5} \end{cases}$$

$$\boldsymbol{CARES_{12}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 6} \\ \mathbf{2} & \boldsymbol{if\ r \geq 6} \end{cases} \qquad \boldsymbol{CARES_{13}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 6} \\ \mathbf{3} & \boldsymbol{if\ r \geq 6} \end{cases}$$

$$\boldsymbol{CARES_{12}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 6.5} \\ \mathbf{2} & \boldsymbol{if\ r \geq 6.5} \end{cases} \qquad \boldsymbol{CARES_{13}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 6.5} \\ \mathbf{3} & \boldsymbol{if\ r \geq 6.5} \end{cases}$$

SAS software, version 9.4 (SAS Institute, Inc., Cary, North Carolina) was used to run the simulations and for the analysis.

2.2.4 Results

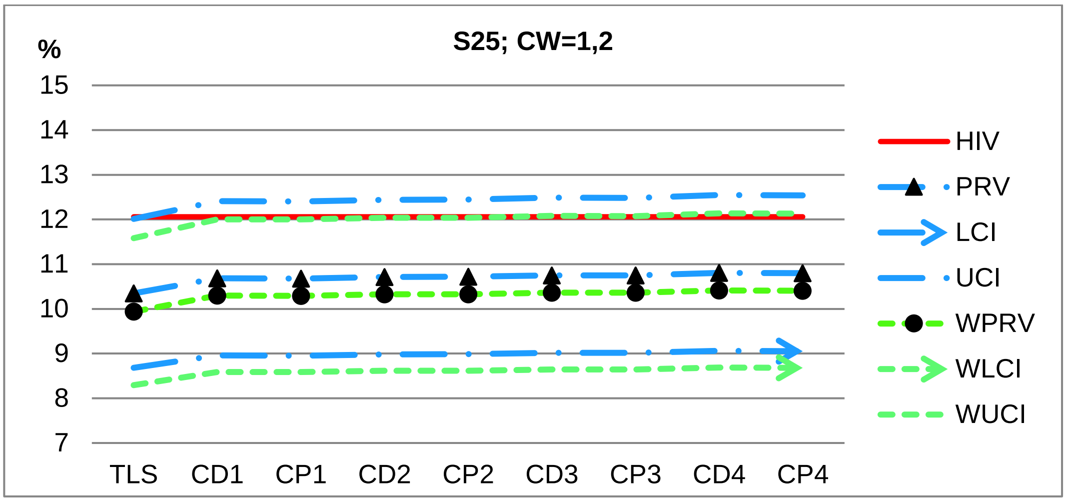
Results are presented in figures below for first stage samples of 25% (S25), 20% (S20) of clusters as an average of 1000 simulations. CARES weights (CW) were applied to samples both with and without the TLS sampling weights in order to make the comparisons possible. The following legend is used in all figures:

TLS	HIV estimate using only TLS
CD1	HIV estimate weighted by CARES on percentile 5 of Deviance residuals
CP1	HIV estimate weighted by CARES on percentile 5 of Pearson residuals
CD2	HIV estimate weighted by CARES on percentile 5.5 of Deviance residuals
CP2	HIV estimate weighted by CARES on percentile 5.5 of Pearson residuals
CD3	HIV estimate weighted by CARES on percentile 6 of Deviance residuals
CP3	HIV estimate weighted by CARES on percentile 6 of Pearson residuals
CD4	HIV estimate weighted by CARES on percentile 6.5 of Deviance residuals
CP4	HIV estimate weighted by CARES on percentile 6.5 of Pearson residuals
HIV	HIV prevalence in the population
PRV	HIV estimates without TLS sampling weights
WPRV	HIV estimates with TLS sampling weights
LCI	Lower confidence interval of estimates without TLS sampling weights
WLCI	Lower confidence interval of estimates with TLS sampling weights
UCI	Upper confidence interval of estimates without TLS sampling weights
WUCI	Upper confidence interval of estimates with TLS sampling weights

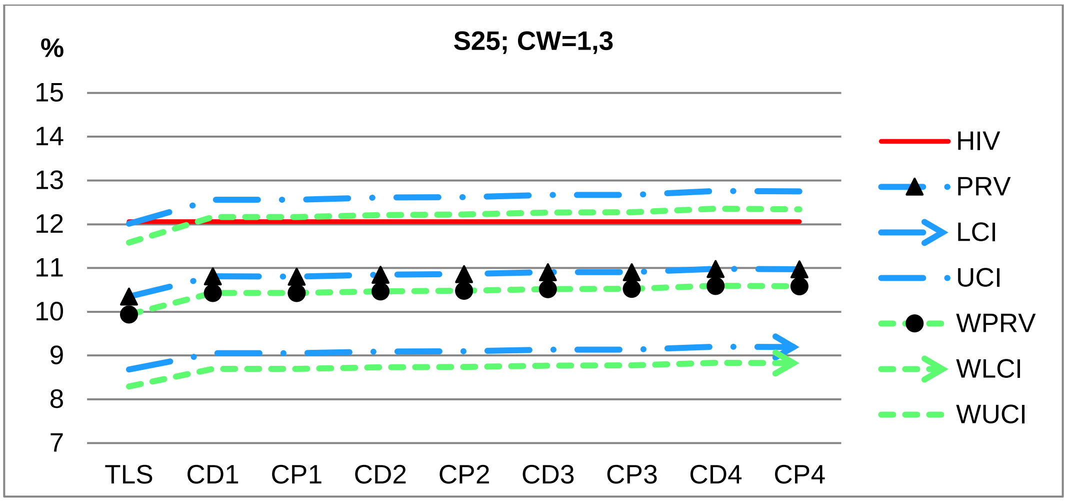
Figure 1 shows the results when pseudo-population 1 (HIV prevalence= 9.89%) was used as the basis for the TLS simulations.

Fig. 1 a) Results for pseudo-population 1 when sampling S25 and CW=1, 2. b) Results for pseudo-population 1 when sampling S25 and CW=1, 3. c) Results for pseudo-population 1 when sampling S20 and CW=1, 2. d) Results for pseudo-population 1 when sampling S20 and CW=1, 3.

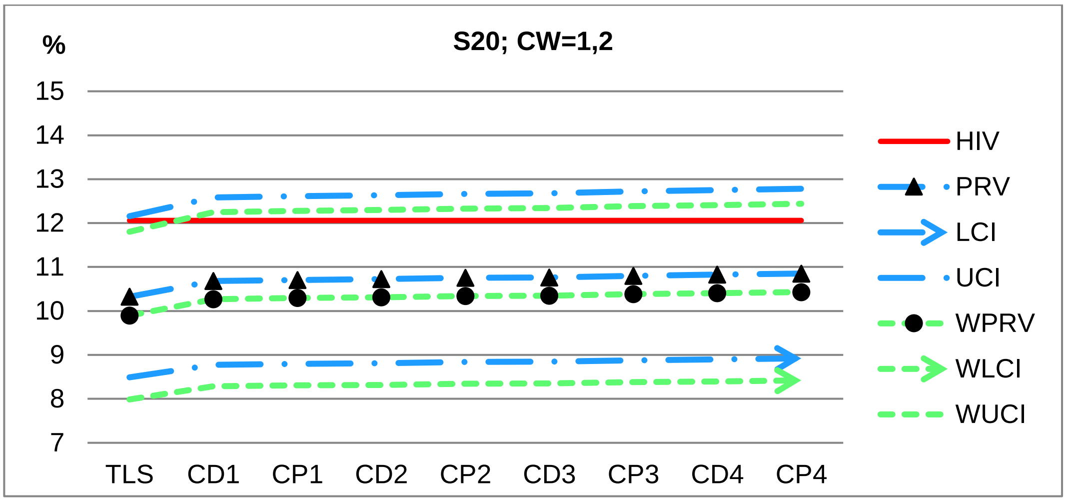
a)


S25; CW=1,2
%
15
14
13
12
11
10
9
8
7
TLS
CD1
CP1
CD2
CP2
CD3
CP3
CD4
CP4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

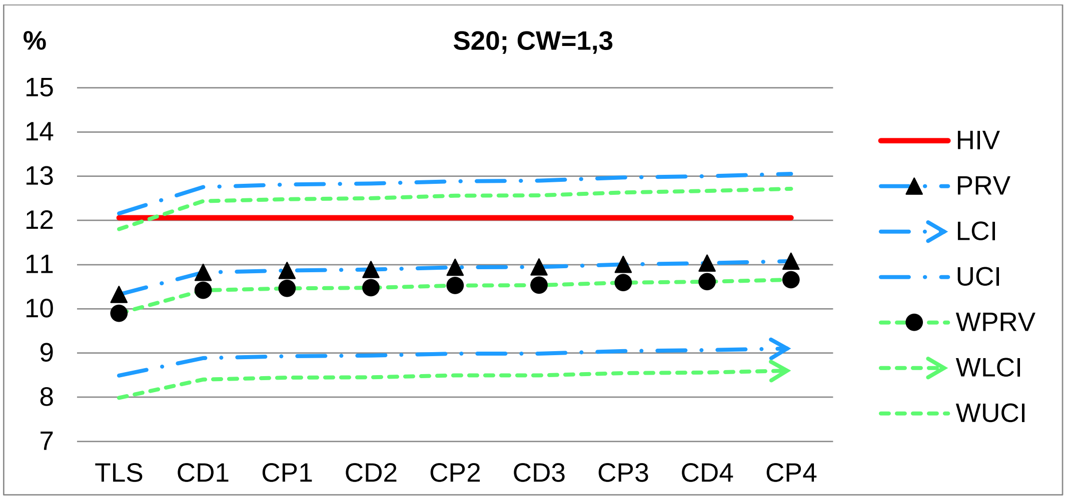
b)


S25; CW=1,3
%
15
14
13
12
11
10
9
8
7
TLS
CD1
CP1
CD2
CP2
CD3
CP3
CD4
CP4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

c)


S20; CW=1,2
%
15
14
13
12
11
10
9
8
7
TLS
CD1
CP1
CD2
CP2
CD3
CP3
CD4
CP4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

d)



Results from sampling S25 and S20 of the clusters from pseudo-population 1 were not significantly different. HIV estimated prevalence, standard errors (SE) (results not shown), and confidence intervals (CI) were similar in both sampling sizes, although SE and CI were slightly greater in S20 results.

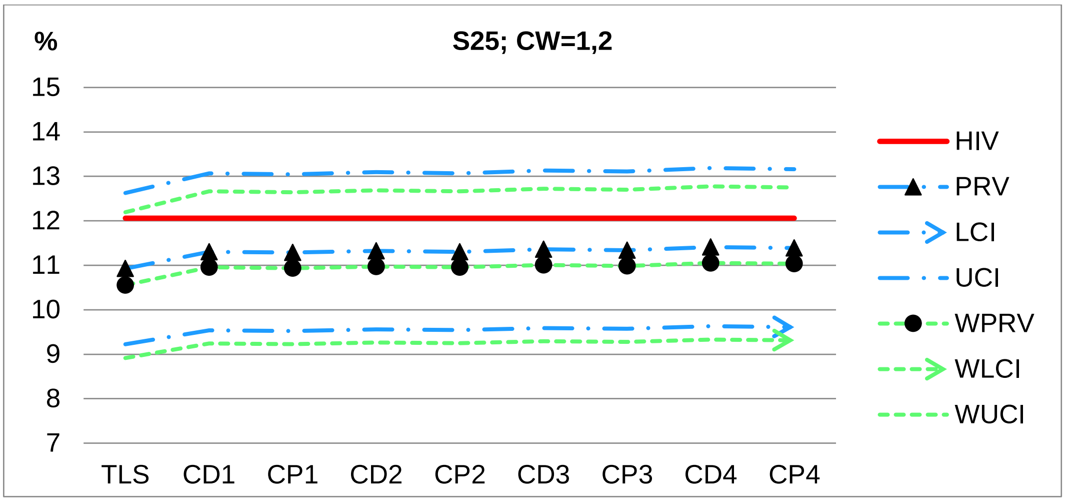
Results for S25 using CW=1, 2 showed that applying CARES weights improved the TLS HIV estimates, bringing them closer to the population prevalence. We can also see that the estimates improved as the percentile of residues to which the CW=2 was applied increased. For both weighted (WPRV in the figures) and unweighted (PRV in the figures) CARES estimates performed about 3%-4% better than TLS. When CW=1, 3 were used estimates also improved for both weighted and unweighted estimates, and in this case CARES estimates were about 4%-5% better than TLS.

Results for S20 were similar to S25. Using CW=1, 2 improved the estimates about 3%-4%, depending on the percentile of residues to which the CW=2 was applied. Additionally, when CW=1, 3 were used estimates also improved for both weighted and unweighted results, and CARES gave about 4%-5% better results than TLS.

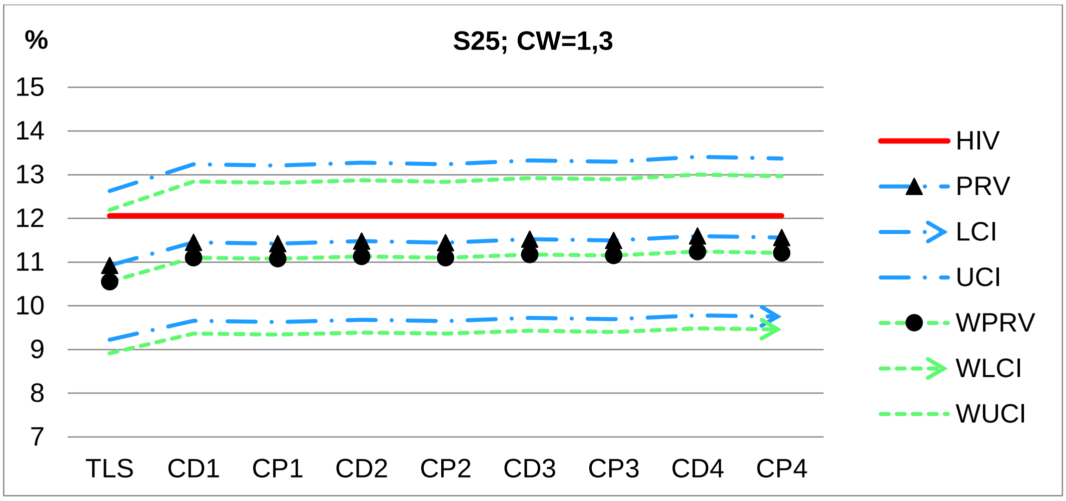
For S25 and S20 there were no significant differences in results when using Deviance or Pearson residuals. Nevertheless, when using pseudo-population1, the best performance was given by CD4 (PRV), CD4 (WPRV) for S25 and CP4 (PRV), CP4 (WPRV) for S20.

Fig. 2 a) Results for pseudo-population 2 when sampling S25 and CW=1, 2. b) Results for pseudo-population 2 when sampling S25 and CW=1, 3. c) Results for pseudo-population 2 when sampling S20 and CW=1, 2. d) Results for pseudo-population 2 when sampling S20 and CW=1, 3.

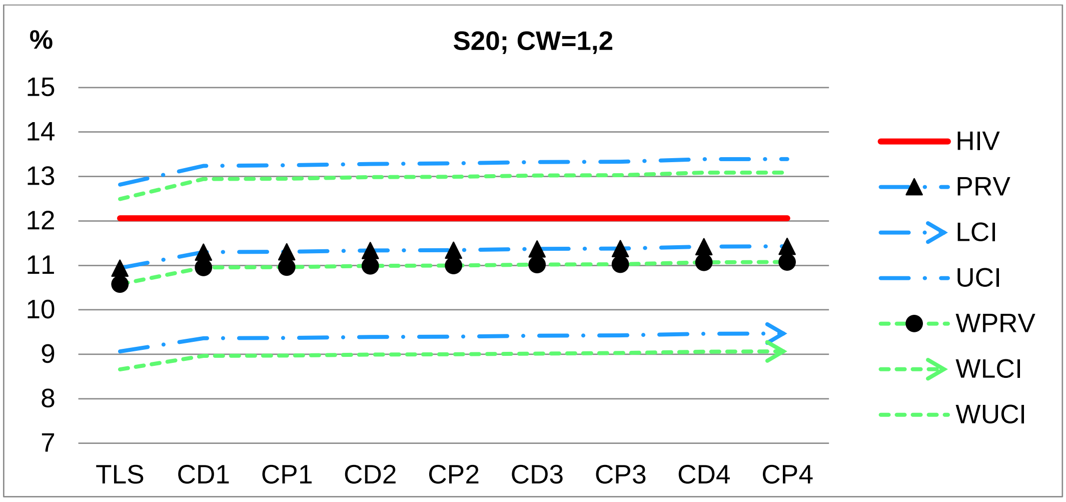
a)



b)



c)



d)

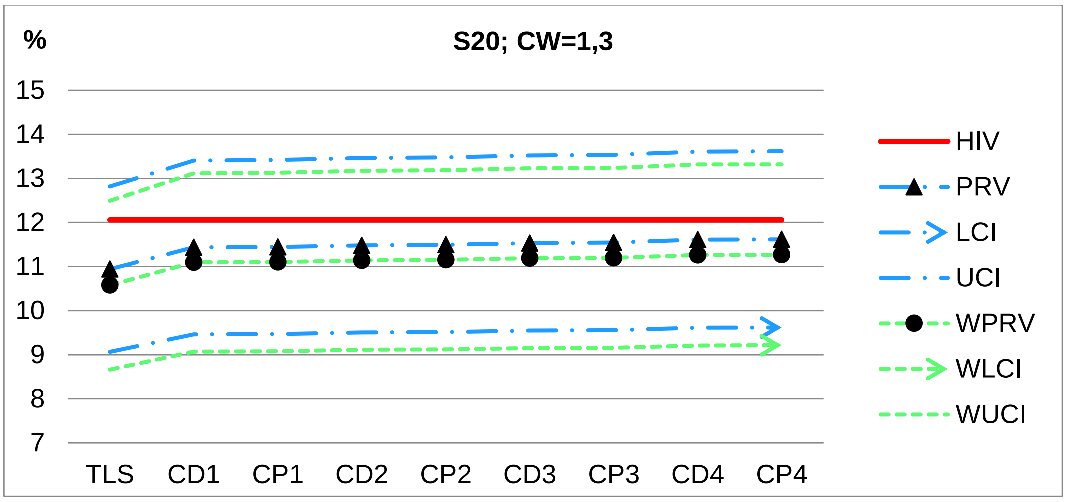


Figure 2 shows that results obtained for pseudo-population 2 were similar to those obtained for pseudo-population 1. HIV estimated prevalence, SE, and CI were similar for S20 and S25, although SE and CI were slightly greater for S20.

Results for S25 using CW=1, 2 also showed that applying CARES weights improved the HIV estimates. CARES estimates improved as the percentile of residues to which the CW=2 was applied increased as well. For both weighted and unweighted estimates CARES performed about 3%-4% better than TLS only. When CW=1, 3 were used

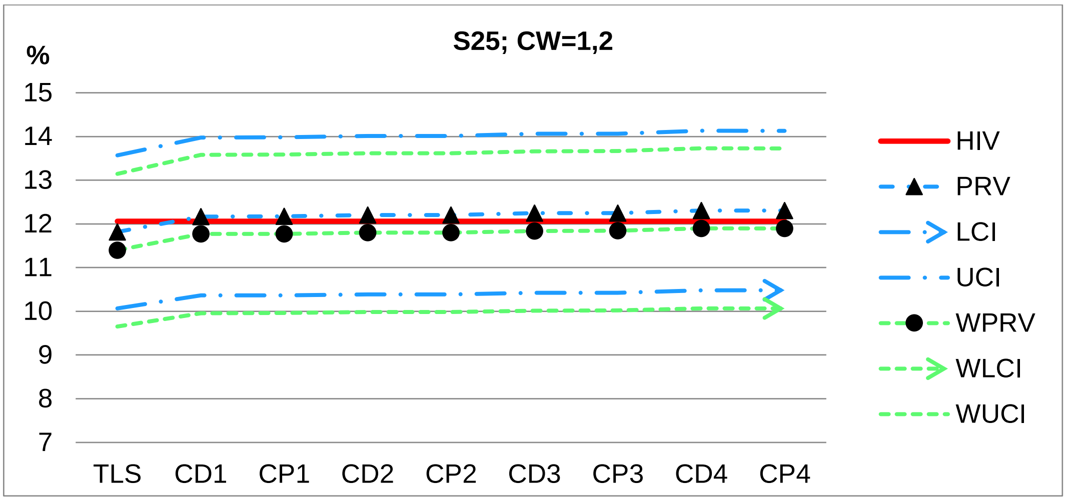
CARES estimates also improved about 4%-6% better than TLS only, for both weighted and unweighted estimates.

Results for S20 were similar to S25; applying CW=1, 2 improved the HIV estimates. Also in this case, the estimates improved as the percentile of residues to which the CW=2 was applied increased, and for both weighted and unweighted estimates CARES performed about 3%-4% better than TLS. Additionally, when CW=1, 3 were applied, CARES estimates also improved for both weighted and unweighted estimates, and were about 4%-6% better than TLS.

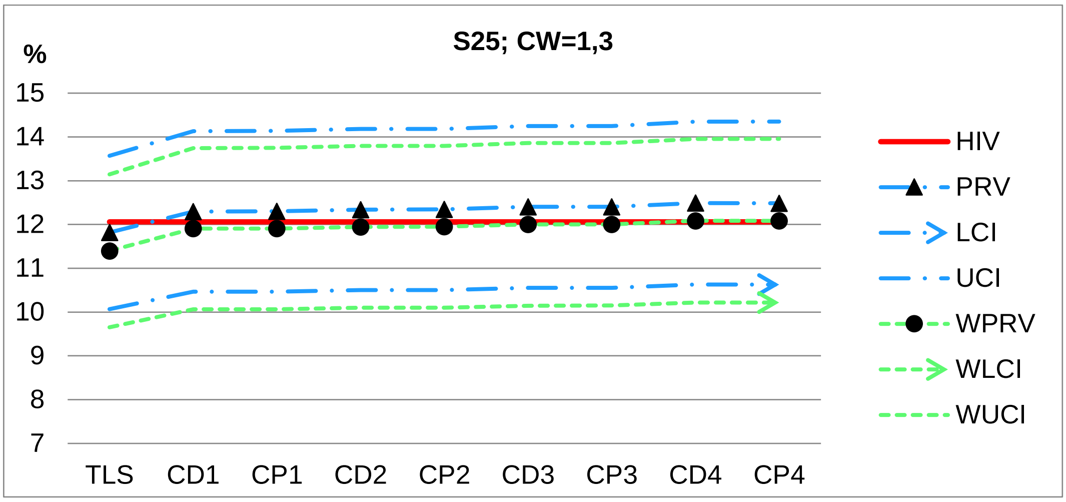
For both S25 and S20, there were no significant differences in results when using Deviance or Pearson residuals. Nevertheless, when using pseudo-population 2, the best performance was given by CD4 (PRV), CD4 (WPRV) for S25 and CP4 (PRV), CP4 (WPRV) for S20.

Fig. 3 a) Results for pseudo-population 3 when sampling S25 and CW=1, 2. b) Results for pseudo-population 3 when sampling S25 and CW=1, 3. c) Results for pseudo-population 3 when sampling S20 and CW=1, 2. d) Results for pseudo-population 3 when sampling S20 and CW=1, 3.

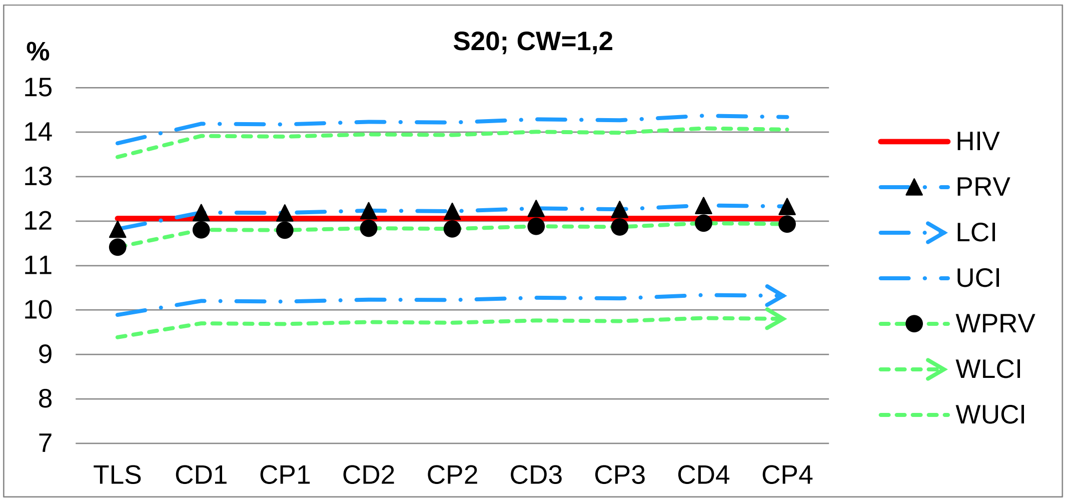
a)



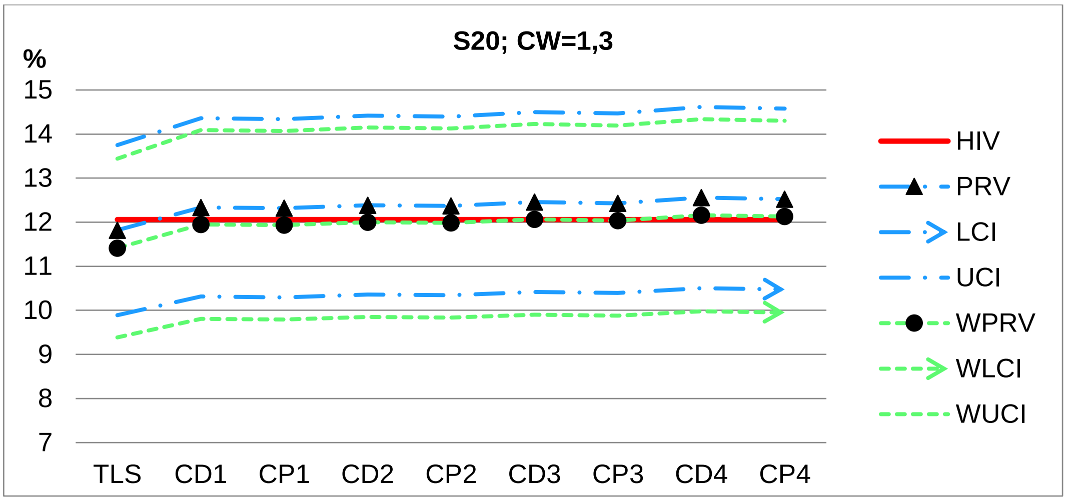
b)


S25; CW=1,3
%
15
14
13
12
11
10
9
8
7
TLS
CD1
CP1
CD2
CP2
CD3
CP3
CD4
CP4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

c)


S20; CW=1,2
%
15
14
13
12
11
10
9
8
7
TLS
CD1
CP1
CD2
CP2
CD3
CP3
CD4
CP4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

d)


S20; CW=1,3
%
15
14
13
12
11
10
9
8
7
TLS
CD1
CP1
CD2
CP2
CD3
CP3
CD4
CP4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

Also, when pseudo-population 3 was used, results for sampling S25 and S20 of the clusters were not significantly different. HIV estimated prevalence, SE, and CI were similar in both cases as in the previous simulations.

Results for S25 using CW=1, 2 showed that applying CARES weights also improved the TLS HIV estimates. We can also see that the estimates improved as the percentile of residues to which the CW=2 was applied increased. However, in this case unweighted results overestimated the HIV prevalence in the study population. In absolute terms the overestimated results were slightly closer to the HIV prevalence in the study population than the TLS HIV estimate, except for the results for CD4 and CP4. Weighted CARES results were below the HIV prevalence in the study population and gave about 3%-4% better estimates than weighted TLS HIV estimates. When CW=1, 3 were applied all unweighted CARES results also overestimated the HIV prevalence in the study population and, in absolute terms, results were no better than TLS HIV estimates. Concerning weighted results, CARES did not overestimate the HIV prevalence (except CP4 and CD4). All CARES results gave about 4%-5% better estimates than weighted TLS HIV estimates.

Results for S20 were similar to S25 and applying CW=1, 2 improved the TLS HIV estimates. Also in this case, CARES results for unweighted estimates overestimated the HIV prevalence in the study population. In absolute terms the overestimated results were slightly closer to the HIV prevalence in the study population than the TLS HIV estimate, except for the results for CD4 and CP4. Weighted CARES results remained below the HIV prevalence in the study population and gave about 3%-4% better estimates than weighted TLS HIV estimates. Additionally, when CW=1, 3 were applied all unweighted CARES estimates overestimated the HIV prevalence in the study population and results were no better than TLS HIV estimates. Concerning weighted results, CARES overestimated the HIV prevalence in the study population, although in absolute terms the differences were lower than TLS estimates.

In this case no significant differences were found in using Deviance or Pearson residuals. The best unweighted results for pseudo-population 3 were given by CD1 and CP1 for S25 and S20 for CW=1, 2 and CW=1, 3. The best weighted results when using

CW=1, 2 were CD4 and CP4 for both S25 and S20, and when using CW=1, 3 the best results were given by CP4 and CD4 for S25, and CP3 and CD3 for S20.

2.2.6 Discussion

Over the last decades several sampling methods have been developed and applied to MARPs to improve estimates of HIV and other communicable diseases, such as Snowball sampling, [31] Respondent Driven Sampling (RDS),[13] Internet sampling,[23] and TLS.[21]

Here we report a new method that improves estimates of HIV prevalence when the TLS technique is used to survey most-at-risk populations.

TLS is useful to collect information about populations who congregate at identifiable places, and through a formative research phase ensures that a high proportion of venues attended by MARPs are included in the sampling frame. TLS randomly selects the venues as proxies for randomly selected population members and it allows making inferences about the population, although a true probability sample of the MARPs is impossible to obtain.[15] Because TLS collects information only about persons who congregate at physical venues and some MARPs may not be reached there,[32] TLS may not reach the most hidden populations or populations with higher risk behaviors.[33] For these reasons, HIV estimates obtained through this method may differ from the real MARPs HIV prevalence.

Our method was tested assuming several scenarios: three pseudo-populations with different levels of HIV prevalence, and two first-stage cluster sampling, with and without sampling weights. We believe that the CARES method can improve the estimated HIV prevalence obtained by the TLS method. CARES make use of Deviance and/or Pearson residuals obtained through logistic regression to calibrate the TLS HIV prevalence estimates. Results show that there were no significant differences in using Deviance or Pearson residuals to assign the CARES weights. Additionally, results from selecting 25% of the clusters and 20% of the clusters were similar for the HIV prevalence, albeit with the last ones obtaining, although only slightly, wider confidence intervals and standard errors as expected.[34] Although several weighting methods have been proposed for TLS[16–18,21] its use remains under discussion or is ignored.[20,21] Some argue that this is because key outcomes might not be associated with venues.[15,19] In our simulation results based on unweighted estimates were more accurate than the weighted ones. Additionally, CARES assigns different weights to individuals depending on the percentile their residues belong to and results show that our method improves both the weighted and unweighted TLS HIV prevalence estimates for all pseudo-populations created.

Imputing weight=1 to lower percentiles and weight=2 or 3 to remaining percentiles, the CARES method assigns a weight=1 to individuals with the lower risk behaviors and gives more weight to those who have higher risk behavior and, consequently, overestimates the results obtained by the TLS method. Concerning this simulation work, the CARES method with weights=1 applied on the percentiles $z < 6.5$ and weight=2 applied on the percentiles $z \geq 6.5$ provided the most balanced results. This choice is a compromise between improving HIV estimates and the risk of obtaining too overestimated results when the estimated HIV prevalence is close to the real one. Using this option, the CARES method improved the HIV estimates in all three pseudo-populations, and in the case of overestimated results on pseudo-population 3, did not worsen the results given by TLS.

Simulation studies have the benefit of knowing the *true* population parameters, although in reality this is not the case and the real HIV prevalence will hardly be known. The great advantage of working with simulations is that we can test several methods/options and assess their performance, always knowing the *true* parameter and drive the efforts to come as close as possible to it. Working in this way obviously we must start with some premises and, in this case, we assumed that the HIV prevalence is underestimated in MARPs, as previously reported.[35,36] We are aware that the CARES method must be further developed before it can be applied but we believe this to be a valuable first step toward a new approach that might help to improve estimates used in planning public health prevention campaigns and tailoring health interventions.

Limitations

This is a new method proposed to improve HIV estimates given by the TLS and consequently has several limitations. We acknowledge that our analysis is too simplistic and a more complex analysis should be developed. The CARES method was compared with traditional TLS HIV prevalence samples, unweighted and weighted by the sampling weights but should also be applied and compared with more complex TLS weighting schemes such as accounting for frequency of venue attendance. We also acknowledge that we cannot prove that our method always performs better than TLS. For this simulation we used a database obtained through an Internet survey. Some studies suggest that some Internet MARPs respondents' risk behaviors might be different from those of MARPs respondents sampled using other recruitment techniques and, for this reason, our results might be biased. This method is only adequate if the disease under study is underestimated. Further research is needed to apply the method to other disease conditions. For this study we selected seven risk factors associated with HIV prevalence present in the EMIS-2010 database and well established in the literature. We do not know if results might have been different had other independent variables been included.

2.2.7 Conclusion

The method proposed to improve TLS HIV estimates provides a means to obtain more accurate results when surveying key populations. This method makes use of logistic regression by assigning different weights to individuals considering their residuals' percentile. We have demonstrated by simulation that the CARES method improves TLS HIV estimates considering populations with different HIV prevalence levels and when using a PPS sampling approach. We hope that this method is recognizable to others and encourages further investigation. New tools are needed to make it more accurate and reliable to use whenever it is not possible to use methods that are more inclusive than the TLS method.

The CARES method can be applied using standard software, and results are easily interpreted.

Acknowledgments

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Supplemental material: results are available from the author

2.2.8 Appendix: Computational Software

Using Pearson residuals and weights=1, 2:

data sample; set sample; if STDRESCHI<Perc_P_5 then weight=1; else weight=2; finalweight=samplingweight*weight;

data sample; set sample; if STDRESCHI<Perc_P_5_5 then weight=1; else weight=2; finalweight=samplingweight*weight;

data sample; set sample; if STDRESCHI<Perc_P_6 then weight=1; else weight=2; finalweight=samplingweight*weight;

data sample; set sample; if STDRESCHI<Perc_P_6_5 then weight=1; else weight=2; finalweight=samplingweight*weight;

Using Deviance residuals and weights=1, 2:

data sample; set sample; if STDRESDEV<Perc_D_5 then weight=1; else weight=2; finalweight=samplingweight*weight;

data sample; set sample; if STDRESDEV<Perc_D_5_5 then weight=1; else weight=2; finalweight=samplingweight*weight;

data sample; set sample; if STDRESDEV<Perc_D_6 then weight=1; else weight=2; finalweight=samplingweight*weight;

data sample; set sample; if STDRESDEV<Perc_D_6_5 then weight=1; else weight=2; finalweight=samplingweight*weight;

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2.2.10 Annex

ANNEX

OF

Improving underestimation of HIV prevalence in surveys using Time-Location Sampling

Ana B Barros, Maria Rosario O Martins

			Method	**HIV**	**SE**	**LCI**	**UCI**
Pseudo-population 1; 25%; 0.7	Weights 1, 2	TLS unweight	TLS	0.1035	0.0085	0.0868	0.1201
			CD1	0.1068	0.0088	0.0896	0.1241
			CP1	0.1068	0.0088	0.0895	0.1240
			CD2	0.1071	0.0088	0.0898	0.1244
			CP2	0.1072	0.0088	0.0899	0.1245
			CD3	0.1075	0.0088	0.0902	0.1249
			CP3	0.1075	0.0088	0.0901	0.1248
			CD4	0.1080	0.0089	0.0906	0.1255
			CP4	0.1080	0.0089	0.0906	0.1254
		TLS weighted	TLS	0.0994	0.0078	0.0829	0.1158
			CD1	0.1030	0.0081	0.0859	0.1200

			CP1	0.1029	0.0080	0.0859	0.1200
			CD2	0.1033	0.0081	0.0861	0.1204
			CP2	0.1033	0.0081	0.0862	0.1204
			CD3	0.1036	0.0081	0.0864	0.1208
			CP3	0.1036	0.0081	0.0864	0.1208
			CD4	0.1041	0.0081	0.0869	0.1214
			CP4	0.1041	0.0081	0.0868	0.1213
	Weights 1, 3	TLS unweight	TLS	0.1035	0.0085	0.0868	0.1201
			CD1	0.1081	0.0089	0.0906	0.1256
			CP1	0.1081	0.0089	0.0905	0.1256
			CD2	0.1085	0.0090	0.0909	0.1261
			CP2	0.1086	0.0090	0.0910	0.1262
			CD3	0.1090	0.0090	0.0914	0.1267
			CP3	0.1091	0.0090	0.0914	0.1267
			CD4	0.1098	0.0091	0.0920	0.1276
			CP4	0.1097	0.0091	0.0919	0.1275
		TLS weighted	TLS	0.0994	0.0078	0.0829	0.1158
			CD1	0.1043	0.0082	0.0869	0.1217
			CP1	0.1043	0.0082	0.0869	0.1216
			CD2	0.1047	0.0082	0.0873	0.1221
			CP2	0.1048	0.0082	0.0874	0.1222
			CD3	0.1052	0.0083	0.0877	0.1227

			CP3	0.1052	0.0082	0.0878	0.1227
			CD4	0.1059	0.0083	0.0883	0.1236
			CP4	0.1059	0.0083	0.0883	0.1234

			Method	**HIV**	**SE**	**LCI**	**UCI**
Pseudo-population 2; 25%; 0.7	Weights 1, 2	TLS unweight	TLS	0.1093	0.0087	0.0922	0.1263
			CD1	0.1130	0.0090	0.0954	0.1307
			CP1	0.1129	0.0090	0.0952	0.1305
			CD2	0.1133	0.0090	0.0956	0.1309
			CP2	0.1130	0.0090	0.0954	0.1307
			CD3	0.1136	0.0090	0.0959	0.1313
			CP3	0.1134	0.0090	0.0957	0.1311
			CD4	0.1141	0.0091	0.0963	0.1319
			CP4	0.1139	0.0091	0.0961	0.1316
		TLS weighted	TLS	0.1055	0.0077	0.0891	0.1219
			CD1	0.1095	0.0081	0.0924	0.1266
			CP1	0.1094	0.0080	0.0923	0.1264
			CD2	0.1097	0.0081	0.0926	0.1269
			CP2	0.1095	0.0081	0.0925	0.1266
			CD3	0.1101	0.0081	0.0929	0.1272
			CP3	0.1099	0.0081	0.0927	0.1270

			CD4	0.1105	0.0081	0.0933	0.1278
			CP4	0.1103	0.0081	0.0931	0.1275
	Weights 1, 3	TLS unweight	TLS	0.1093	0.0087	0.0922	0.1263
			CD1	0.1145	0.0091	0.0966	0.1324
			CP1	0.1142	0.0091	0.0963	0.1321
			CD2	0.1148	0.0092	0.0968	0.1328
			CP2	0.1144	0.0091	0.0965	0.1324
			CD3	0.1152	0.0092	0.0972	0.1333
			CP3	0.1150	0.0092	0.0970	0.1329
			CD4	0.1160	0.0092	0.0978	0.1341
			CP4	0.1156	0.0092	0.0975	0.1337
		TLS weighted	TLS	0.1055	0.0077	0.0891	0.1219
			CD1	0.1110	0.0082	0.0936	0.1284
			CP1	0.1108	0.0082	0.0934	0.1281
			CD2	0.1113	0.0082	0.0939	0.1288
			CP2	0.1110	0.0082	0.0936	0.1284
			CD3	0.1118	0.0082	0.0943	0.1292
			CP3	0.1115	0.0082	0.0940	0.1289
			CD4	0.1124	0.0083	0.0948	0.1300
			CP4	0.1121	0.0083	0.0946	0.1297

			Method	HIV	SE	LCI	UCI
Pseudo-population 3; 25%; 0.7	Weights 1, 2	TLS unweight	TLS	0.1182	0.0089	0.1007	0.1357
			CD1	0.1217	0.0092	0.1036	0.1398
			CP1	0.1217	0.0092	0.1036	0.1398
			CD2	0.1220	0.0092	0.1039	0.1401
			CP2	0.1220	0.0092	0.1039	0.1401
			CD3	0.1224	0.0093	0.1043	0.1406
			CP3	0.1225	0.0093	0.1043	0.1406
			CD4	0.1231	0.0093	0.1048	0.1413
			CP4	0.1231	0.0093	0.1048	0.1413
		TLS weighted	TLS	0.1140	0.0082	0.0965	0.1314
			CD1	0.1177	0.0085	0.0996	0.1358
			CP1	0.1177	0.0086	0.0996	0.1359
			CD2	0.1180	0.0086	0.0998	0.1361
			CP2	0.1180	0.0086	0.0998	0.1362
			CD3	0.1184	0.0086	0.1002	0.1366
			CP3	0.1184	0.0086	0.1002	0.1366
			CD4	0.1190	0.0086	0.1007	0.1373
			CP4	0.1190	0.0086	0.1007	0.1373
	Weights 1, 3	TLS unweight	TLS	0.1182	0.0089	0.1007	0.1357
			CD1	0.1230	0.0093	0.1046	0.1413
			CP1	0.1230	0.0094	0.1047	0.1414

			CD2	0.1234	0.0094	0.1050	0.1418
			CP2	0.1234	0.0094	0.1050	0.1418
			CD3	0.1240	0.0094	0.1055	0.1425
			CP3	0.1240	0.0094	0.1056	0.1425
			CD4	0.1249	0.0095	0.1063	0.1435
			CP4	0.1249	0.0095	0.1063	0.1435
		TLS weighted	TLS	0.1140	0.0082	0.0965	0.1314
			CD1	0.1190	0.0087	0.1006	0.1375
			CP1	0.1191	0.0087	0.1007	0.1375
			CD2	0.1194	0.0087	0.1010	0.1379
			CP2	0.1195	0.0087	0.1010	0.1380
			CD3	0.1200	0.0088	0.1015	0.1386
			CP3	0.1200	0.0088	0.1015	0.1386
			CD4	0.1209	0.0088	0.1022	0.1395
			CP4	0.1209	0.0088	0.1022	0.1395

			Method	**HIV**	**SE**	**LCI**	**UCI**
Pseudo-population 1; 20%; 0.7	Weights 1, 2	TLS unweight	TLS	0.1032	0.0093	0.0849	0.1216
			CD1	0.1068	0.0097	0.0878	0.1258
			CP1	0.1071	0.0097	0.0880	0.1261
			CD2	0.1072	0.0097	0.0881	0.1263

			CP2	0.1075	0.0098	0.0884	0.1267
			CD3	0.1076	0.0098	0.0885	0.1268
			CP3	0.1080	0.0098	0.0888	0.1272
			CD4	0.1083	0.0098	0.0890	0.1275
			CP4	0.1085	0.0098	0.0892	0.1278
		TLS weighted	TLS	0.0989	0.0088	0.0799	0.1180
			CD1	0.1027	0.0092	0.0828	0.1225
			CP1	0.1029	0.0092	0.0831	0.1228
			CD2	0.1031	0.0092	0.0832	0.1230
			CP2	0.1034	0.0092	0.0835	0.1233
			CD3	0.1035	0.0092	0.0835	0.1234
			CP3	0.1038	0.0093	0.0838	0.1238
			CD4	0.1041	0.0093	0.0840	0.1241
			CP4	0.1043	0.0093	0.0842	0.1244
	Weights 1, 3	TLS unweight	TLS	0.1032	0.0093	0.0849	0.1216
			CD1	0.1082	0.0099	0.0889	0.1276
			CP1	0.1087	0.0099	0.0893	0.1281
			CD2	0.1089	0.0099	0.0894	0.1283
			CP2	0.1094	0.0099	0.0898	0.1289
			CD3	0.1094	0.0100	0.0899	0.1290
			CP3	0.1101	0.0100	0.0904	0.1297
			CD4	0.1103	0.0100	0.0906	0.1300

			CP4	0.1108	0.0101	0.0910	0.1305
		TLS weighted	TLS	0.0989	0.0088	0.0799	0.1180
			CD1	0.1042	0.0093	0.0840	0.1243
			CP1	0.1046	0.0094	0.0844	0.1248
			CD2	0.1048	0.0094	0.0845	0.1250
			CP2	0.1052	0.0094	0.0849	0.1256
			CD3	0.1053	0.0094	0.0849	0.1257
			CP3	0.1059	0.0095	0.0855	0.1263
			CD4	0.1061	0.0095	0.0856	0.1267
			CP4	0.1066	0.0095	0.0860	0.1272

			Method	**HIV**	**SE**	**LCI**	**UCI**
Pseudo-population 2; 20%; 0.7	Weights 1, 2	TLS unweight	TLS	0.1094	0.0096	0.0906	0.1282
			CD1	0.1130	0.0099	0.0936	0.1324
			CP1	0.1131	0.0099	0.0936	0.1325
			CD2	0.1133	0.0099	0.0939	0.1328
			CP2	0.1134	0.0099	0.0940	0.1329
			CD3	0.1137	0.0099	0.0942	0.1332
			CP3	0.1138	0.0100	0.0943	0.1333
			CD4	0.1143	0.0100	0.0946	0.1339
			CP4	0.1143	0.0100	0.0947	0.1339

		TLS weighted	TLS	0.1058	0.0089	0.0866	0.1249
			CD1	0.1095	0.0092	0.0896	0.1294
			CP1	0.1096	0.0092	0.0897	0.1295
			CD2	0.1099	0.0092	0.0899	0.1298
			CP2	0.1100	0.0092	0.0900	0.1299
			CD3	0.1102	0.0093	0.0902	0.1302
			CP3	0.1103	0.0093	0.0903	0.1303
			CD4	0.1107	0.0093	0.0906	0.1308
			CP4	0.1108	0.0093	0.0907	0.1309
	Weights 1, 3	TLS unweight	TLS	0.1094	0.0096	0.0906	0.1282
			CD1	0.1143	0.0100	0.0946	0.1340
			CP1	0.1145	0.0101	0.0947	0.1342
			CD2	0.1148	0.0101	0.0950	0.1346
			CP2	0.1150	0.0101	0.0952	0.1348
			CD3	0.1153	0.0101	0.0955	0.1352
			CP3	0.1155	0.0101	0.0956	0.1353
			CD4	0.1161	0.0102	0.0961	0.1361
			CP4	0.1162	0.0102	0.0962	0.1362
		TLS weighted	TLS	0.1058	0.0089	0.0866	0.1249
			CD1	0.1109	0.0094	0.0907	0.1312
			CP1	0.1110	0.0094	0.0908	0.1313
			CD2	0.1114	0.0094	0.0911	0.1317

			CP2	0.1115	0.0094	0.0912	0.1319
			CD3	0.1119	0.0094	0.0915	0.1323
			CP3	0.1120	0.0094	0.0916	0.1324
			CD4	0.1126	0.0095	0.0921	0.1331
			CP4	0.1127	0.0095	0.0922	0.1332

			Method	**HIV**	**SE**	**LCI**	**UCI**
Pseudo-population 3; 20%; 0.7	Weights 1, 2	TLS unweight	TLS	0.1182	0.0098	0.0989	0.1375
			CD1	0.1220	0.0102	0.1020	0.1419
			CP1	0.1218	0.0102	0.1019	0.1418
			CD2	0.1223	0.0102	0.1023	0.1423
			CP2	0.1222	0.0102	0.1022	0.1422
			CD3	0.1228	0.0102	0.1028	0.1429
			CP3	0.1226	0.0102	0.1026	0.1427
			CD4	0.1235	0.0103	0.1034	0.1437
			CP4	0.1233	0.0103	0.1032	0.1434
		TLS weighted	TLS	0.1141	0.0094	0.0939	0.1344
			CD1	0.1180	0.0098	0.0970	0.1391
			CP1	0.1179	0.0097	0.0969	0.1390
			CD2	0.1184	0.0098	0.0973	0.1395
			CP2	0.1183	0.0098	0.0972	0.1394

			CD3	0.1189	0.0098	0.0976	0.1401
			CP3	0.1187	0.0098	0.0975	0.1399
			CD4	0.1195	0.0099	0.0982	0.1409
			CP4	0.1193	0.0099	0.0980	0.1406
	Weights 1, 3	TLS unweight	TLS	0.1182	0.0098	0.0989	0.1375
			CD1	0.1234	0.0103	0.1031	0.1436
			CP1	0.1232	0.0103	0.1030	0.1434
			CD2	0.1239	0.0104	0.1036	0.1442
			CP2	0.1237	0.0103	0.1034	0.1440
			CD3	0.1246	0.0104	0.1042	0.1450
			CP3	0.1243	0.0104	0.1039	0.1447
			CD4	0.1256	0.0105	0.1050	0.1461
			CP4	0.1252	0.0105	0.1047	0.1457
		TLS weighted	TLS	0.1141	0.0094	0.0939	0.1344
			CD1	0.1195	0.0099	0.0981	0.1409
			CP1	0.1193	0.0099	0.0979	0.1407
			CD2	0.1200	0.0100	0.0985	0.1415
			CP2	0.1198	0.0099	0.0984	0.1413
			CD3	0.1206	0.0100	0.0990	0.1423
			CP3	0.1204	0.0100	0.0988	0.1420
			CD4	0.1216	0.0101	0.0998	0.1434
			CP4	0.1213	0.0101	0.0995	0.1430

2.3 Calibration of HIV prevalence estimates in most-at-risk populations: results from a two countries simulation study

2.3.1 Abstract

On a previous study we presented a method to improve HIV estimates when TLS method was used to survey key-populations. In this paper we want to confirm CARES performance by applying it to two real country databases, the Portuguese with 3720 men who have sex with men (MSM) and an HIV prevalence of 10.8% and the Spanish with 9591 MSM and an HIV prevalence of 12.1%. We used these prevalences as a proxy of the countries' real prevalence. For each country database we simulated TLS populations (called pseudo-populations) and from each pseudo-population we simulated 1000 TLS samples. The estimated HIV prevalence obtained using only the TLS method to survey the population and using the CARES method were recorded and compared with the HIV prevalence of the respective country. Regardless of the countries' HIV prevalence estimates, results showed that CARES method improved the TLS HIV estimates.

2.3.2 Background

Most-at-risk populations (MARPs) also called hidden or hard-to reach populations, are defined as those populations at highest risk for sexual acquisition/transmission of communicable diseases such has HIV and are frequently associated with stigmatized or illegal behaviors.[1] Examples of MARPs are men who have sex with men (MSM), injection drug users (IDU), prisoners and homeless people.[2] Therefore, accurate estimates for HIV on MARPs are essential to track the course of the epidemics however, these estimates are not easy to obtain for two major reasons: first, most studies are based on self-reported data and in these situations the HIV prevalence tends to be underestimated[3,4] and second, it is hard to get reliable estimates because sampling frames of these populations do not exist[1,5] and consequently probabilistic methods can hardly be used. To overcome this difficulty, several non-probabilistic and semi-probabilistic methods have been developed and used.[1] One of those methods is time-location-sampling (TLS), also called time-space sampling or venue-based sampling.

TLS is a semi-probabilistic method that consists in identifying the venues (bars, night-clubs, saunas, etc.), and time periods (blocks of time are constructed depending the number of hours a venue is open, e.g.: every 4 hour is a block) where the study population congregates and then select a sample of sites to recruit members during a pre-defined time interval.[6] Combination of location and time is known as a sample event (also called cluster or primary sampling unit). After identifying the venues, investigators randomly choose a sample of them (usually a simple random sample without replacement) and then, in each venue, select a sample of persons from among those appearing to meet the eligibility criteria (e.g.: if the study concerns female sex workers, researchers must approach women that appear to be sex workers). The number of attendees that appear belonging to the study population present on the venue is also registered to build the sampling weights.[6–8] TLS is an efficient way to sample hidden populations that congregate in specific locations and is able to, theoretically, approximate probability sampling [8] however not all selected places are easily accessible and others are not even contemplated due to safety reasons or high costs.[9] Besides, populations that congregate at public venues may differ from the true population as some of them may only frequent private venues.[10,11] This means that there might be an unknown potential bias in the estimates.[12] For these reasons, proposals to improve the estimates obtained through TLS method continue to appear on a regular basis.[11,13,14] On a previous research[15] we proposed a method to improve estimates of the HIV prevalence on MARPs when TLS is used to collect the information. The method, CARES (*calibration on residuals*), calibrates the TLS HIV prevalence estimates using the logistic regression residuals. Using the Spanish sub-sample of the EMIS-2010 survey we run a computationally intensive simulation and found that the proposed method worked well when we assume that the HIV prevalence is underestimated. Because we only used a unique country database, in this paper we want to confirm the CARES results by applying the method to both Portuguese and Spanish databases and compare the outputs. The goal of this study is therefore to assess CARES performance in other population with different level of the HIV prevalence and different sampling sizes. The performance of the method CARES is then compared between the two countries.

2.3.3 Methods

Populations

Portuguese and Spanish databases provided by the European MSM Internet Survey (EMIS-2010) were used to run the simulations. The purpose of EMIS was to develop a pan-European Internet survey on HIV to identify sexual behaviours and prevention needs of European MSM.[16] The project was developed with participating countries' leads. International MSM dating websites were contacted to send instant messages to their members to answer an on-line survey. Data was collected between June and August 2010 and 174,209 individuals from 38 countries answered in an eligible manner to the questionnaire.[16] Additional details of the EMIS survey can be found elsewhere.[16,17] Respondents who self-identified as men, who answered ever received an HIV test and reported the test result were selected for the study-populations.

Spanish study-population has 9591 individuals and the corresponding HIV prevalence is 12.1% and we will use this prevalence as a proxy to the real prevalence on the Spanish population. Respondents identified 19 geographical regions as the place where they were living. Those who did not report the region were they were living were excluded. Regions with fewer than 150 respondents were also excluded because the TLS method tends to include in a survey physical venues where many participants congregate because the probability of selecting respondents is greater.[6] Additionally, about 25% of the study population was intentionally left out (randomly selected) because TLS method can only access key-populations who frequent known venues. The decision to withdraw from the study 25% of the study population was empirical because we did not find any literature that could shed light on the coverage rate of key-populations by the TLS method neither the population size using venue-based methods.[18] Additionally, 135 respondents who reported being HIV positive were randomly selected and excluded from de database because we are assuming that results obtained for HIV prevalence are underestimated.[3,19] Therefore the Spanish pseudo-population used as basis to run the simulations had 6057 individuals distributed by 10 geographical regions and an HIV prevalence of 9.9%. This pseudo-population is the Spanish population we assumed frequent physical venues where participants congregate (it is the supposed population identified by the formative research if the TLS method was indeed used).

Portuguese study-population has 3720 individuals and an HIV prevalence of 10.8%. Also in this case, we will use this prevalence as a proxy to the real prevalence on the Portuguese MSM population. Respondents identified 20 geographical regions as the place where they were living. Respondents who did not report the region were they were living were excluded. Due to size constraints, the following procedure was applied. Regions with fewer than 30 respondents were also excluded because the TLS method tends to include in a survey physical venues where many participants congregate because the probability of selecting respondents is greater, as reported above.[6] Additionally, 50 respondents who reported being HIV positive were randomly selected and excluded from de database in order to have an underestimated HIV prevalence. The Portuguese pseudo-population used as basis to run the simulations had 3160 individuals distributed by 12 geographical regions and an HIV prevalence of 9.2%. This pseudo-population is the Portuguese population we assumed frequent physical venues where participants congregate (is the supposed population identified by the formative research if the TLS method was indeed used).

Sampling

The TLS method was simulated using the two-stage sampling design approch.[6,8,20] The question “which region do you live in?” included in the EMIS survey questionnaire was used to create the sample events (venues or clusters) and one time interval.[20]

In each Spanish geographical region, respondents were randomly allocated to venues. Regions with fewer respondents were assigned at least two venues and regions with more respondents were assigned a maximum of 13 venues. Spanish pseudo-population was therefore divided in 68 venues, ranging from 29 to 166 persons randomly allocated by venue and considering that the number of persons by venue should be proportional to the number of respondents in the respective region.

In each Portuguese geographical region, respondents were randomly allocated to venues. Between one and six clusters were assigned to each region except the one region where about 50% of all respondents reported living, to which were assigned 13 clusters. Portuguese pseudo-population was therefore divided in 44 venues ranging from

34 to 114 persons randomly allocated by venue and also considering that the number of persons by venue should be proportional to the number of respondents in the region.

For each country, samples were drawn using the TLS method in accordance with the following description: in the first stage, the sampling design probability proportional to size (PPS) was used to select 30% (20 and 13 venues were selected from Spanish and Portuguese databases respectively) and 25% (17 and 11 venues were selected from Spanish and Portuguese databases respectively) of the venues and on the second stage was assumed that about 70% of the respondents from each selected venue answered the questionnaire.[20] The number of persons in the venue at the interview time was assumed to be known in order to determine the sampling weights. For each pseudo-population and for each first stage sample size 1000 samples were drawn. The prevalence of HIV infection was recorded weighted and unweighted as a mean of each 1000 samples. From now on HIV prevalence estimates calculated using the TLS method will be written as TLS HIV estimates. Standard errors and confidence intervals were also recorded.

The method

Details of the method have been previously described[15]. Briefly, the method calibrates the TLS HIV prevalence estimates using the logistic regression residuals. Logistic regression model is widely used in cases where the outcome variable is binary or dichotomous.[21]

In logistic regression there are several possible ways to measure the difference between the observed and fitted values. We used the two most common types of measures. The first type is called the Pearson residual, and is based on the idea of subtracting off the mean and dividing by the standard deviation:

$$\boldsymbol{r(y_i, \hat{\pi}_i) = \frac{(y_i - n_i\hat{\pi}_i)}{\sqrt{n_i\hat{\pi}_i(1-\hat{\pi}_i)}}} \qquad \text{(Eq 1)}$$

where n_i subjects share the ith covariate pattern and y_i of them experience the event of interest. Combining the Pearson residuals produces the *Pearson chi-square statistic*: $X^2 = \sum r_i^2$

The second type is the deviance residual:

$$d(y_i, \hat{\pi}_i) = \pm\sqrt{2\left[y_i ln\left(\frac{y_i}{n_i\hat{\pi}_i}\right) + (n_i - y_i) ln\left(\frac{(n_i - y_i)}{n_i(1-\hat{\pi}_i)}\right)\right]} \quad \text{(Eq 2)}$$

where the sign is positive when $y_i \geq \hat{\pi}_i$ and negative otherwise. The summary statistic based on deviance residuals is the deviance: $D = \sum d_i^2$

CARES method consists in assigning different weights to individuals according to the percentile their residuals belong and the weights are distributed as follows:

$$CARES = \begin{cases} a & if\ r < z \\ b & if\ r \geq z \end{cases} \quad \text{(Eq 3)}$$

where a and b are the weights, r represents the residuals, and z is the percentile of the residuals.

Simulation

Similar to the previous study the simulations were made in the following way. For each TLS sample drawn, a logistic regression model was run with HIV (positive/negative) as the outcome variable and “age”(up to 24 years/25-49 years/50 years or more) “having a relationship with a steady partner”(yes/no), “had STI symptoms in the last 12 months”(yes/no), “age at first anal intercourse”(up to 15 years/16 or more), “had unprotected anal intercourse the last time had sex” (yes/no), “ever taken illicit drugs” (yes/no) and “education level”(low/mid/high) as covariates for HIV prevalence in MSM population according to the literature.[22–24] The stepwise regression method and a significance level of the chi-square score of 0.1 were used for entering variables into the model. Pearson and Deviance logistic regression residuals were recorded as standardized values. The method was tested with several weights applied to different percentiles of residues in order to compare the performances. The ones described below presented the best results:

$$CARES_{12} = \begin{cases} 1 & if\ r < 5 \\ 2 & if\ r \geq 5 \end{cases} \qquad CARES_{13} = \begin{cases} 1 & if\ r < 5 \\ 3 & if\ r \geq 5 \end{cases}$$

$$CARES_{12} = \begin{cases} 1 & if\ r < 5.5 \\ 2 & if\ r \geq 5.5 \end{cases} \qquad CARES_{13} = \begin{cases} 1 & if\ r < 5.5 \\ 3 & if\ r \geq 5.5 \end{cases}$$

$$\boldsymbol{CARES_{12}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 6} \\ \mathbf{2} & \boldsymbol{if\ r \geq 6} \end{cases} \qquad \boldsymbol{CARES_{13}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 6} \\ \mathbf{3} & \boldsymbol{if\ r \geq 6} \end{cases}$$

$$\boldsymbol{CARES_{12}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 6.5} \\ \mathbf{2} & \boldsymbol{if\ r \geq 6.5} \end{cases} \qquad \boldsymbol{CARES_{13}} = \begin{cases} \mathbf{1} & \boldsymbol{if\ r < 6.5} \\ \mathbf{3} & \boldsymbol{if\ r \geq 6.5} \end{cases}$$

SAS software, version 9.4 (SAS Institute, Inc., Cary, North Carolina) was used to run the simulations and for the analysis.

2.3.4 Results

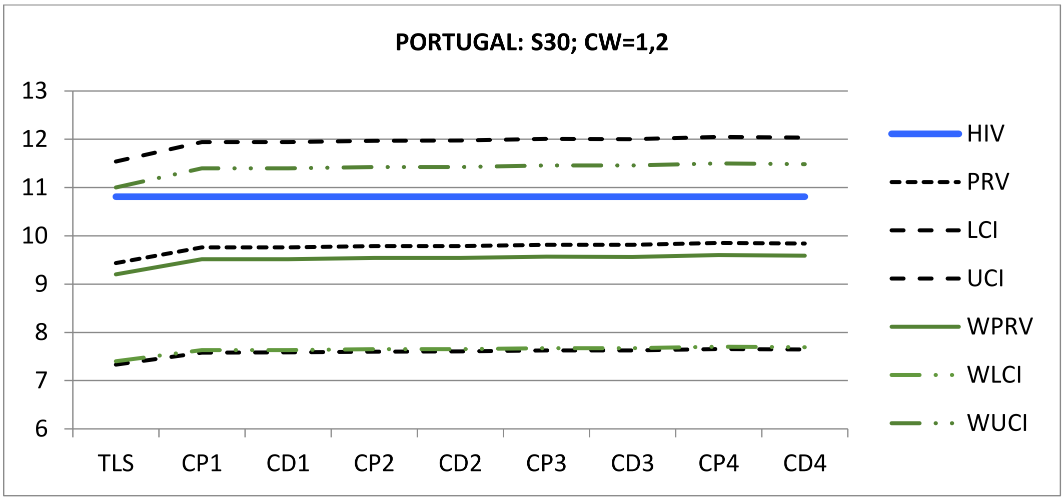
Results are presented in figures below when sampling 30% (S30) and 25% (S25) of the venues as an average of 1000 simulations. CARES weights (CW) were applied both to crude (unweighted) and weighted (by the sampling weights) HIV TLS results in order to make the comparisons possible. The following legend is used in all figures:

HIV	HIV prevalence in the population
TLS	HIV estimate using only TLS
CD1	HIV estimate weighted by CARES on percentile 5 of Deviance residuals
CP1	HIV estimate weighted by CARES on percentile 5 of Pearson residuals
CD2	HIV estimate weighted by CARES on percentile 5.5 of Deviance residuals
CP2	HIV estimate weighted by CARES on percentile 5.5 of Pearson residuals
CD3	HIV estimate weighted by CARES on percentile 6 of Deviance residuals
CP3	HIV estimate weighted by CARES on percentile 6 of Pearson residuals
CD4	HIV estimate weighted by CARES on percentile 6.5 of Deviance residuals
CP4	HIV estimate weighted by CARES on percentile 6.5 of Pearson residuals
PRV	HIV TLS unweighted estimates
WPRV	HIV TLS weighted estimates
LCI	Lower confidence interval of estimates without TLS weights
WLCI	Lower confidence interval of estimates with TLS weights
UCI	Upper confidence interval of estimates without TLS weights
WUCI	Upper confidence interval of estimates with TLS weights

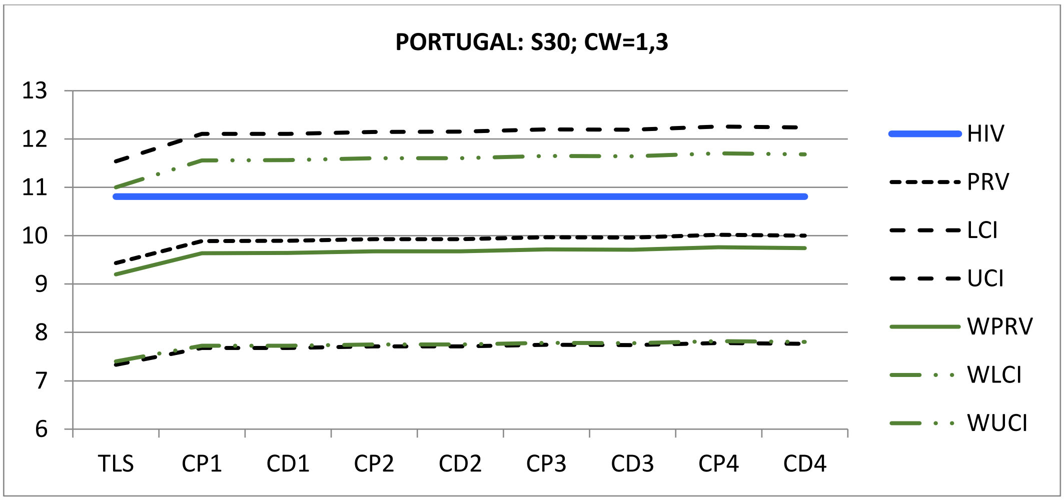
Figure 1 shows the results when sampling 30% of the venues for both Portuguese and Spanish databases.

Fig. 1 a) Portugal, CW=1, 2, (in%). b) Portugal, CW=1, 3 (in%). c) Spain, CW=1, 2 (in%). d) Spain, CW=1, 3 (in%)

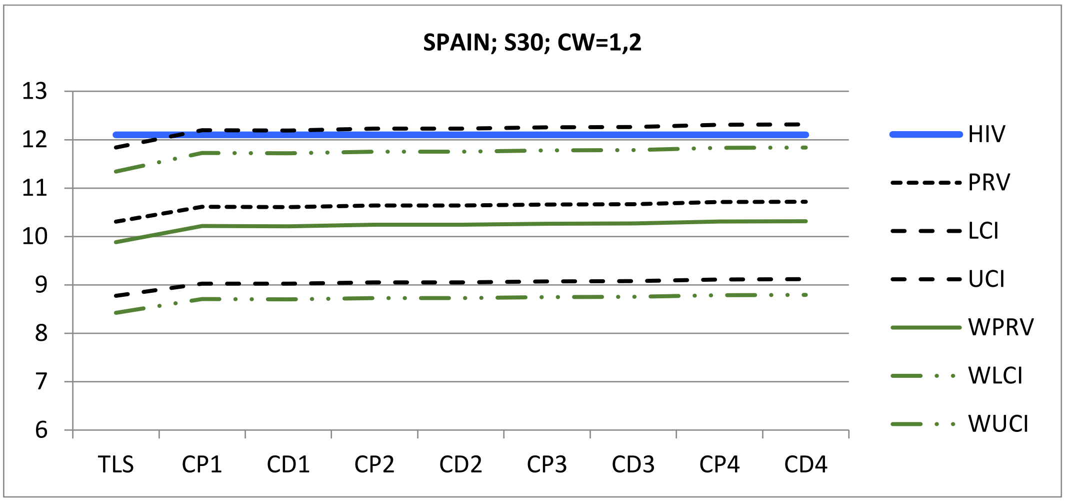
a)



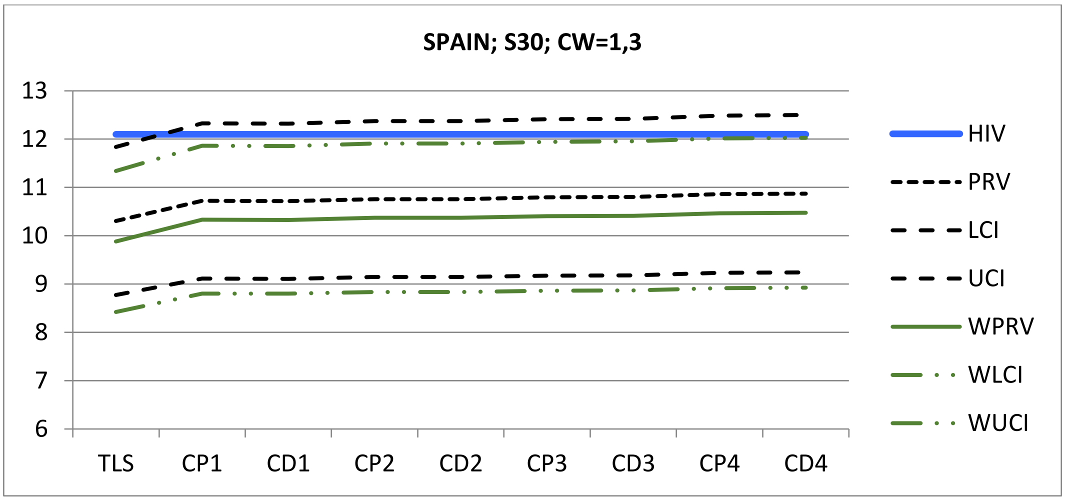
b)



c)



d)



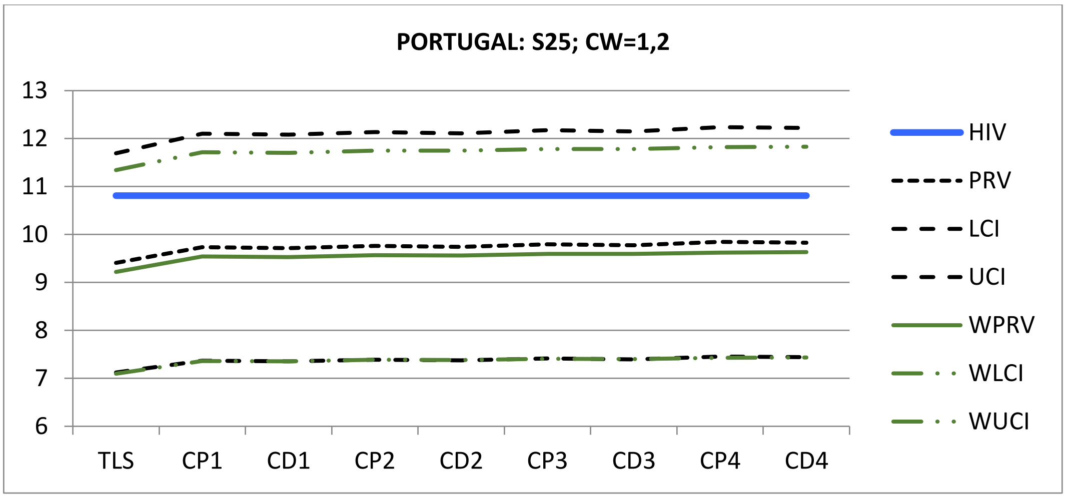
CARES method performed equally well for both countries' database. For all simulated conditions, CARES method provided better estimates than HIV TLS estimates for both unweighted results (PRV on the figures) and weighted results (WPRV on the figures). Moreover, unweighted results provided estimates closer to the population prevalence than weighted results. As the percentile to which weight=1 was applied increased, results approximated to the populations' prevalence for both CW=1, 2 and even more close when CW=1, 3. When CW=1, 2 were applied, the HIV TLS estimates improved between 3%-4% for both countries, and about 4%-5% when CW=1, 3 were used. Portuguese results produced slightly greater standard errors (SE) than the Spanish ones.

For both countries, simulations produced larger SE in unweighted samples than in weighted samples, though this difference is minimal. The amplitude of confidence intervals (CI) was greater on the Portuguese case than on the Spanish case and for both countries the amplitude was bigger for unweighted than for weighted data. There were no significant differences in results when using Deviance or Pearson residuals.

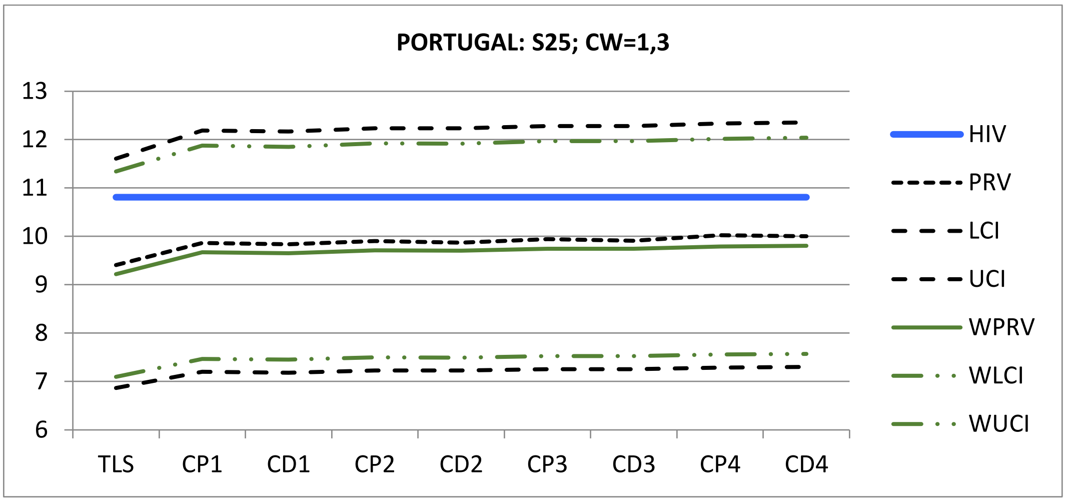
Figure 2 shows the results when sampling 25% of the venues using both Portuguese and Spanish databases.

Fig. 2 a) Portugal, CW=1, 2, (in%). b) Portugal, CW=1, 3 (in%). c) Spain, CW=1, 2 (in%). d) Spain, CW=1, 3 (in%)

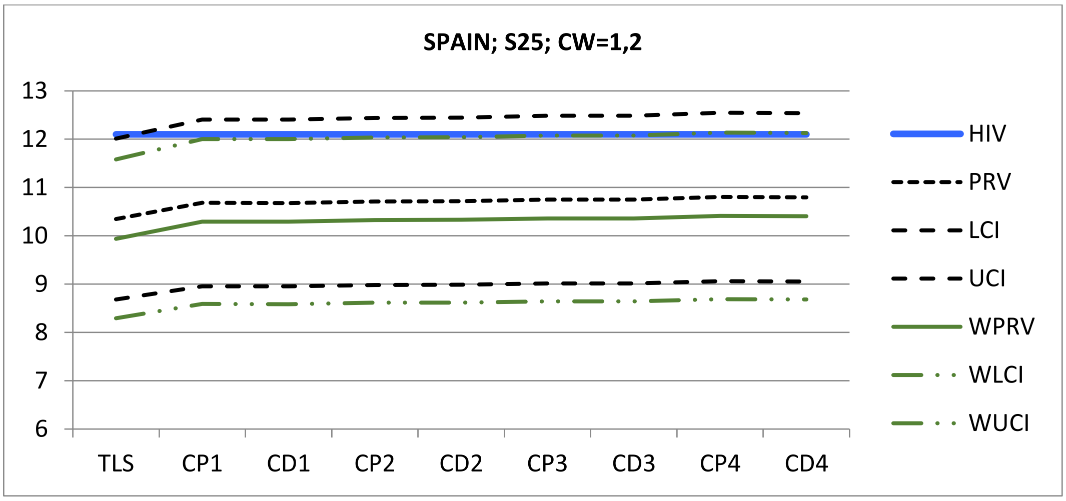
a)



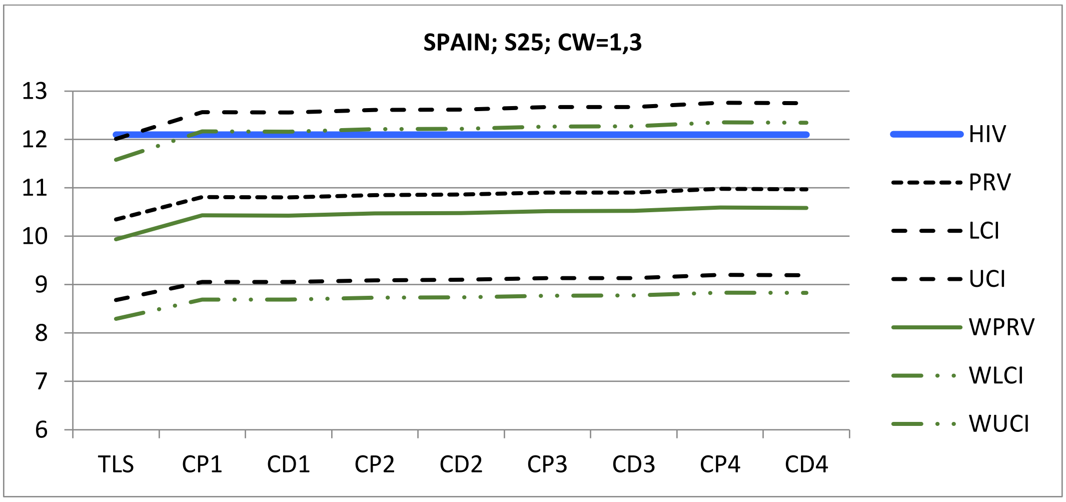
b)


PORTUGAL: S25; CW=1,3
13
12
11
10
9
8
7
6
TLS
CP1
CD1
CP2
CD2
CP3
CD3
CP4
CD4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

c)


SPAIN; S25; CW=1,2
13
12
11
10
9
8
7
6
TLS
CP1
CD1
CP2
CD2
CP3
CD3
CP4
CD4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

d)


SPAIN; S25; CW=1,3
13
12
11
10
9
8
7
6
TLS
CP1
CD1
CP2
CD2
CP3
CD3
CP4
CD4
HIV
PRV
LCI
UCI
WPRV
WLCI
WUCI

In similar way, simulation results when 25% of the venues were sampled, CARES method performed equally well for both countries' database. Also in this case, and for all simulated conditions, CARES method provided better estimates than when using only HIV TLS estimates for both unweighted and weighted results. Unweighted results provided estimates closer to the population prevalence than weighted results. As the percentile to which weight=1 was applied increased, results approximated to the populations' prevalence for both CW=1, 2 and CW=1, 3. When CW=1, 2 were applied, the HIV TLS estimates improved between 3%-4% for both countries, and about 4%-5% when CW=1, 3. Portuguese data produced slightly greater SE than Spanish data. And for both countries, simulations produced larger SE in unweighted samples than in weighted samples, though this difference is minimal. The amplitude of CI was greater on the Portuguese case than on the Spanish case and for both countries this amplitude was greater for unweighted than for weighted data. There were no significant differences in results when using Deviance or Pearson residuals.

Though differences are minimal, results from sampling 25% of the venues produced larger SE and wider CI for both countries than when sampling 30%.

2.3.5 Discussion

In the last decades there has been a growing concern to determine as accurately as possible the prevalence of communicable diseases in MARPs. Methods used to survey those populations have evaluated since the most basic "convenience" sampling method until the more sophisticated TLS or respondent driven sampling (RDS).[11] However, even the most recent methods are subject to bias and therefore are always subject to proposals for their improvement.[11,25]

In this paper we intended to confirm CARES performance as a way to improve HIV estimates obtained through the TLS method. This new proposed method, CARES, had already been presented on a previous research and results were promising. Here we applied the method using two different countries' database.

Portugal and Spain have different levels of HIV prevalence and they were used to assess if the method performed equally well. Results showed that CARES improved the estimated HIV prevalence obtained by the TLS method in both countries' dataset, confirming in this way that the method performs well.

Results showed that there were no significant differences in using Deviance or Pearson residuals to assign the CARES weights. Additionally, results of selecting 30% and 25% of the venues were similar for the estimated HIV prevalence though the last ones had wider confidence intervals and standard errors as expected. [26]

Similar to the previous study, also in this case unweighted TLS HIV prevalence was more accurate than the weighted TLS HIV prevalence for both countries. CARES weights performed equally well in both countries' database for both weighted and unweighted estimates, though slightly better on weighted ones.

Weighting TLS is an attempt to reduce bias and its use remains under discussion, some defending its use and proposing ways to improve [8,13,14,27] and others ignoring them[11,14] with the argument that key outcomes might not be associated with venues.[6,28]

CARES assigns different weights to individuals depending on the percentile their residues belong and results confirm that our method improves the weighted and unweighted TLS HIV prevalence estimates for both countries' database. CARES method gives weight=1 to respondents of lower percentiles of residues and weight=2 or 3 to the remaining. This means that the method assigns a weight=1 to HIV negative individuals with low risk behaviours and gives more weight to the others. As the percentile to which weight=1 is given increases, results come closer to the (proxy) real HIV prevalence, this means that the method is giving weight=1 to more low-risk individuals.

In this study, we assumed that both Portuguese and Spanish pseudo-populations have an underestimated HIV prevalence of more than 1% when compared to the respective study-populations HIV prevalence and results of CW=1,3 performed better than results of CW=1,2. Consequently, for both Portuguese and Spanish databases, the best results were given for CP4 and CD4 for both weighted and unweighted. Nevertheless this is not always the case, as shown on the previous article, because there is the danger of getting too overestimated results when the estimated HIV prevalence is close to the real one[15].

Working with the simulations allows us to test several scenarios and methods and compare them with a (known) *true* parameter though this might differ from reality. This work confirmed CARES' good performance. CARES method still is at an embryonic stage and must be further developed before can be applied. Nevertheless we believe we are on the right direction to establish a new method for improving the HIV estimates obtained when TLS is used to study most-at-risk populations.

Limitations

We identified several limitations. Our analysis is too simple and a more complex one should be developed. We assumed that the HIV prevalence is underestimated. We did not account for the frequency of venue attendance to be included when weighting. Additionally, we assumed all sampling units had equal probability of inclusion within the cluster, which might not be the case in real situations. We used the PPS approach to sample the venues and we do not know how might be the CARES performance if other approach was used. We selected seven risk factors associated with the HIV prevalence present in the EMIS database and well established in the literature. We do not know if results might have been different if other independent variables were included. Databases used in this work are from Internet studies. Respondents' characteristics may differ from the TLS respondents and therefore results might be distorted.

The size of Portuguese database was small for running simulations. Also, the Portuguese HIV prevalence is low. These two characteristics make the logistic regression model fit questionable in samples where the quasi-complete separation of data points occurred therefore CARES results presented here for the Portuguese case might be imprecise. This also means that for small dimension studies and low prevalence diseases, CARES might not be adequate.

2.3.6 Conclusion

CARES is a recently proposed method to improve TLS HIV estimates when surveying hard-to-reach populations. Using two country' databases, we run several simulations and test the method at different levels. TLS samples were drawn and the logistic regression model was applied. CARES method assigns different weights to individuals considering their logistic regression residuals' percentile and the results showed that CARES improved the estimated HIV prevalence obtained by the TLS method in both countries' datasets. We believe this is a further step to validate its adequacy.

2.3.7 References

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2.3.8 Annex

ANNEX

OF

Calibration of HIV prevalence estimates in most-at-risk populations: results from a two countries simulation study

Ana B Barros, Maria Rosario O Martins

			Method	**HIV**	**SE**	**LCI**	**UCI**
Portuguese pseudo-population: 30%; 0.7	Weights 1, 2	TLS unweight	TLS	0.094	0.011	0.073	0.115
			CD1	0.098	0.011	0.076	0.119
			CP1	0.098	0.011	0.076	0.119
			CD2	0.098	0.011	0.076	0.120
			CP2	0.098	0.011	0.076	0.120
			CD3	0.098	0.011	0.076	0.120
			CP3	0.098	0.011	0.076	0.120
			CD4	0.099	0.011	0.077	0.120
			CP4	0.098	0.011	0.076	0.120
		TLS weighted	TLS	0.092	0.008	0.074	0.110
			CD1	0.095	0.009	0.076	0.114
			CP1	0.095	0.009	0.076	0.114
			CD2	0.095	0.009	0.077	0.114

			CP2	0.095	0.009	0.077	0.114
			CD3	0.096	0.009	0.077	0.115
			CP3	0.096	0.009	0.077	0.115
			CD4	0.096	0.009	0.077	0.115
			CP4	0.096	0.009	0.077	0.115
	Weights 1, 3	TLS unweight	TLS	0.094	0.011	0.073	0.115
			CD1	0.099	0.011	0.077	0.121
			CP1	0.099	0.011	0.077	0.121
			CD2	0.099	0.011	0.077	0.121
			CP2	0.099	0.011	0.077	0.122
			CD3	0.100	0.011	0.077	0.122
			CP3	0.100	0.011	0.077	0.122
			CD4	0.100	0.011	0.078	0.123
			CP4	0.100	0.011	0.078	0.122
		TLS weighted	TLS	0.092	0.008	0.074	0.110
			CD1	0.096	0.009	0.077	0.116
			CP1	0.096	0.009	0.077	0.116
			CD2	0.097	0.009	0.077	0.116
			CP2	0.097	0.009	0.077	0.116
			CD3	0.097	0.009	0.078	0.116
			CP3	0.097	0.009	0.078	0.116
			CD4	0.098	0.009	0.078	0.117

			CP4	0.097	0.009	0.078	0.117

			Method	HIV	SE	LCI	UCI
Portuguese pseudo-population: 25%; 0.7	Weights 1, 2	TLS unweight	TLS	0.094	0.012	0.071	0.117
			CD1	0.097	0.012	0.074	0.121
			CP1	0.097	0.012	0.074	0.121
			CD2	0.098	0.012	0.074	0.121
			CP2	0.097	0.012	0.074	0.121
			CD3	0.098	0.012	0.074	0.122
			CP3	0.098	0.012	0.074	0.121
			CD4	0.098	0.012	0.075	0.122
			CP4	0.098	0.012	0.074	0.122
		TLS weighted	TLS	0.092	0.009	0.071	0.113
			CD1	0.095	0.010	0.073	0.117
			CP1	0.095	0.010	0.073	0.117
			CD2	0.095	0.010	0.074	0.117
			CP2	0.095	0.010	0.073	0.117
			CD3	0.096	0.010	0.074	0.117
			CP3	0.095	0.010	0.074	0.117
			CD4	0.096	0.010	0.074	0.118
			CP4	0.096	0.010	0.074	0.118

	Weights 1, 3	TLS unweight	TLS	0.094	0.012	0.071	0.117
			CD1	0.099	0.012	0.075	0.123
			CP1	0.098	0.012	0.074	0.122
			CD2	0.099	0.012	0.075	0.123
			CP2	0.099	0.012	0.075	0.123
			CD3	0.099	0.012	0.075	0.124
			CP3	0.099	0.012	0.075	0.123
			CD4	0.100	0.012	0.076	0.125
			CP4	0.100	0.012	0.076	0.124
		TLS weighted	TLS	0.092	0.009	0.071	0.113
			CD1	0.096	0.010	0.074	0.118
			CP1	0.096	0.010	0.074	0.118
			CD2	0.097	0.010	0.075	0.119
			CP2	0.096	0.010	0.074	0.118
			CD3	0.097	0.010	0.075	0.119
			CP3	0.097	0.010	0.075	0.119
			CD4	0.098	0.010	0.075	0.120
			CP4	0.098	0.010	0.075	0.120

			Method	**HIV**	**SE**	**LCI**	**UCI**
Spanish pseudo-	Weights 1, 2	TLS	TLS	0.103	0.008	0.088	0.118

population: 30%; 0.7		unweight	CD1	0.106	0.008	0.090	0.122
			CP1	0.106	0.008	0.090	0.122
			CD2	0.106	0.008	0.091	0.122
			CP2	0.106	0.008	0.090	0.122
			CD3	0.107	0.008	0.091	0.123
			CP3	0.107	0.008	0.091	0.123
			CD4	0.107	0.008	0.091	0.123
			CP4	0.107	0.008	0.091	0.123
		TLS weighted	TLS	0.099	0.007	0.084	0.113
			CD1	0.102	0.007	0.087	0.117
			CP1	0.102	0.007	0.087	0.117
			CD2	0.102	0.007	0.087	0.118
			CP2	0.102	0.007	0.087	0.118
			CD3	0.103	0.007	0.087	0.118
			CP3	0.103	0.007	0.088	0.118
			CD4	0.103	0.007	0.088	0.118
			CP4	0.103	0.007	0.088	0.118
	Weights 1, 3	TLS unweight	TLS	0.103	0.008	0.088	0.118
			CD1	0.107	0.008	0.091	0.123
			CP1	0.107	0.008	0.091	0.123
			CD2	0.108	0.008	0.091	0.124
			CP2	0.108	0.008	0.091	0.124

			CD3	0.108	0.008	0.092	0.124
			CP3	0.108	0.008	0.092	0.124
			CD4	0.109	0.008	0.092	0.125
			CP4	0.109	0.008	0.092	0.125
		TLS weighted	TLS	0.099	0.007	0.084	0.113
			CD1	0.103	0.007	0.088	0.119
			CP1	0.103	0.007	0.088	0.119
			CD2	0.104	0.007	0.088	0.119
			CP2	0.104	0.007	0.088	0.119
			CD3	0.104	0.007	0.089	0.119
			CP3	0.104	0.007	0.089	0.120
			CD4	0.105	0.007	0.089	0.120
			CP4	0.105	0.007	0.089	0.120

			Method	**HIV**	**SE**	**LCI**	**UCI**
Spanish pseudo-population: 25%; 0.7	Weights 1, 2	TLS unweight	TLS	0.103	0.008	0.087	0.120
			CD1	0.107	0.009	0.090	0.124
			CP1	0.107	0.009	0.090	0.124
			CD2	0.107	0.009	0.090	0.124
			CP2	0.107	0.009	0.090	0.124
			CD3	0.108	0.009	0.090	0.125

			CP3	0.107	0.009	0.090	0.125
			CD4	0.108	0.009	0.091	0.125
			CP4	0.108	0.009	0.091	0.125
		TLS weighted	TLS	0.099	0.008	0.083	0.116
			CD1	0.103	0.008	0.086	0.120
			CP1	0.103	0.008	0.086	0.120
			CD2	0.103	0.008	0.086	0.120
			CP2	0.103	0.008	0.086	0.120
			CD3	0.104	0.008	0.086	0.121
			CP3	0.104	0.008	0.086	0.121
			CD4	0.104	0.008	0.087	0.121
			CP4	0.104	0.008	0.087	0.121
	Weights 1, 3	TLS unweight	TLS	0.103	0.008	0.087	0.120
			CD1	0.108	0.009	0.091	0.126
			CP1	0.108	0.009	0.091	0.126
			CD2	0.109	0.009	0.091	0.126
			CP2	0.109	0.009	0.091	0.126
			CD3	0.109	0.009	0.091	0.127
			CP3	0.109	0.009	0.091	0.127
			CD4	0.110	0.009	0.092	0.128
			CP4	0.110	0.009	0.092	0.127
		TLS	TLS	0.099	0.008	0.083	0.116

		weighted	CD1	0.104	0.008	0.087	0.122
			CP1	0.104	0.008	0.087	0.122
			CD2	0.105	0.008	0.087	0.122
			CP2	0.105	0.008	0.087	0.122
			CD3	0.105	0.008	0.088	0.123
			CP3	0.105	0.008	0.088	0.123
			CD4	0.106	0.008	0.088	0.124
			CP4	0.106	0.008	0.088	0.123

Part III – DISCUSSION AND CONCLUSIONS

DISCUSSION AND CONCLUSIONS

3.1 Summary of findings and discussion

International Health focuses on health-related issues of the world's most vulnerable people namely problems that affects low and middle-income countries (Beaglehole & Bonita, 2010; Koplan et al., 2009).

HIV infection disproportionately affects low and middle-income countries and is the underlying cause of death of about 1 million people every year in the sub-Saharan Africa (Frank et al., 2019). Additionally, HIV infection also disproportionately affects key populations in Africa (Bigna & Nansseu, 2019; Chemaitelly, Weiss, Calvert, Harfouche, & Abu-Raddad, 2019; Stannah et al., 2019) and other middle and low-income countries (WHO, 2019). The Global Health sector strategy on HIV 2016-2021 (WHO, 2016) identifies several areas for fast-track action and one of them is reaching and protecting those most vulnerable and at risk HRP.

At this point International Health faces two challenges: the first is to identify and recruit the HRP and the second is to get accurate HIV prevalence estimates. Both challenges are linked because the more effective the recruitment method the more likely it is to get accurate estimates. However, as already stated in the introduction of this dissertation, all recruitment methods have advantages and disadvantages. Besides, accurate estimates of HIV prevalence in HRP do not depend solely on the recruitment technique it also depends on the interviewee's ability to accurately answer the questions, as also already stated in the introduction section.

This dissertation was based on three research studies. The objective was to develop a method that could improve the accuracy of HIV prevalence estimates obtained when time-location sampling method is used to survey hard-to-reach populations.

Methods used to recruit HRP have long been a concern for the need to get reliable estimates that could be representative of the target populations. Until the early 80s, most research on hidden populations was based on information gathered about individuals on institutional settings (e.g.: clinics, hospitals, prisons) which had limited the generalizability of the findings (Watters & Biernacki, 1989). Although the need to conduct studies outside the institutional settings have been early pointed out (Waldorf & Reinarman, 1975) only in the 80s, with the emergency of HIV, the technique employed on the recruitment of HRP became more significant with the need to provide accurate information to public-health decision makers (Watters & Biernacki, 1989). Since then several authors have highlighted the importance of recruitment methods (e.g.: Faugier & Sargeant, 1997; Magnani, Sabin, Saidel, & Heckathorn, 2005) to obtain reliable information and, in recent years, some methods have been developed to improve its precision (Abdul-Quader, Heckathorn, Sabin, & Saidel, 2006; Leon, Jauffret-Roustide, & Le Strat, 2015; Ramirez-Valles, Heckathorn, Vázquez, Diaz, & Campbell, 2005; Shi, Cameron, & Heckathorn, 2019) as already stated in the introduction of this dissertation.

Aware of the constant evolution that envisage the improvement of recruitment methods, this thesis begun by a systematic literature review of the methods used to recruit HRP of MSM and FSW between 2003-2013.

In this thesis we found that the sampling methods of convenience, snowball, Internet, RDS and TLS were used in 85% of the studies. Our results are consistent with other studies that found convenience sampling (Miller, Buckingham, Sánchez-Domínguez, Morales-Miranda, & Paz-Bailey, 2013), snowball, RDS ((Ellard-Gray, Jeffrey, Choubak, & Crann, 2015), Internet (Noble, Jones, Bowles, DiNenno, & Tregear, 2017) and TLS (Gios et al., 2016) the most frequently used to sample HRP populations.

We found significantly more studies about MSM (more than 80%) than about FSW (less than 20%). In fact, studies conducted on FSW are scarce (Baral et al., 2012; Shannon et al., 2015), a systematic literature review on FSW found that in the United States of America only two studies were conducted between 2003-2013 (Paz-Bailey, Noble, Salo, & Tregear, 2016). Our finding is therefore in accordance with others.

About 68% of MSM studies used semi-probabilistic methods to recruit respondents but FSWs were more frequently recruited by non-probabilistic methods. MSM tend to be network connected and congregate at venues to socialize and met sexual partners (Amirkhanian, 2014), additionally, internet is a very popular way among MSM to seek partners (Martin et al., 2019), therefore semi-probabilistic methods are an efficient way to recruit them. On the other hand, FSW tend to have small network groups, unsupportive relationships with other FSW (Fearon et al., 2019) and sparse external relations, therefore is more difficult to apply a uniform approach to survey them (Dong, Wang, Hu, & Liu, 2019), which might implicate that, in some circumstances, non-probabilistic methods are the best way to reach them.

We also found that the semi-probabilistic methods (Internet, RDS and TLS) recruited more than 80% of the respondents. Internet surveys are an efficient way to recruit a great number of respondents in short time (Hall et al., 2017). Additionally, RDS and TLS can also recruit a great number of respondents, especially when compared with the traditional non-probability sampling techniques (Ellard-Gray et al., 2015; Raymond, Chen, & McFarland, 2019; Valerio et al., 2016; Weinmann et al., 2019). Our results are thus consistent with other findings showing that semi-probabilistic methods are being more used in health research.

An update of the systematic literature review from 2014 to 2019 was recently made on the Pubmed. Pubmed database was selected because it is one of the largest databases in the biomedical literature (National Library of Medicine, 2019).

The key-words used as search terms were on the title and abstract: men who have sex with men or msm or female sex worker and convenience sampling or purposive sampling or snowball sampling or cluster sampling or Internet sampling or respondent-driven sampling or RDS or time-location sampling or TLS. Peer reviewed articles, written in English, and published from 01/01/2014 until 19/12/2019 were the selected criteria. The search identified 428 studies from which 26 were eliminated because they were duplicates (7), reviews (8) or protocols (11). The selected studies are presented in table 1, by population-type and recruitment method.

About 86 studies were identified FSW related and 316 studies were identified MSM related. In a total of 402 studies, 4 studies were both FSW and MSM related.

The number of published articles in the last six years, 2014-2019, is much superior when compared to the number of articles published between 2003-2013 (402 studies and 268 studies respectively).

Similar to the systematic literature review made between 2003-2013, in the last six years FSW are present in about 21% of the retrieved studies and MSM on 79%. Studies concerning FSW continue to be less than MSM studies.

RDS survey method was used in almost 50% of the studies followed by Internet sampling (18.5%) and TLS (8.8%). Also in the last six years, as previous reported, semi-probabilistic methods are the most used (on about 80% of the studies) to survey these key-populations. Of note is that in the last six years FSW are being more frequently recruited by semi-probabilistic methods (58%) than previously (on 2003-2013) 50%, which confirms the tendency of semi-probabilistic methods being increasingly used.

Table 1: Retrieved publications from 2014 until 2019 by population and recruitment method

Population (%)		FSW (%)	MSM(%)	Total(%)
Recruitment methods	Convenience	7 (1.8)	20 (5)	27 (6.8)
	Purposive	8 (2)	8 (2)	16 (4)
	Snowball	12 (3)	25 (6.3)	37 (9.3)
	Cluster	9 (2.3)	5 (1.3)	14 (3.5)
	Internet	0	74 (18.5)	74 (18.5)
	RDS	35 (8.3)	164 (41)	197 (49.3)
	TLS	15 (3.8)	20 (5)	35 (8.8)
	Total	86 (21)	316 (79)	402 (100)

About 30 countries were identified that published studies on FSW (table 2). Countries that produced most FSW related studies were China, (16 studies, about 20%), followed by Zimbabwe (6 studies, about 7.3%) and South Africa (6 studies, about 7.3%). These results are consistent with the previous literature review confirming China as the leading country on FSW research publications. South Africa increased the number of studies from 1 in the period 2003-2013 to 6 in the last six years (2014-2019). Zimbabwe also published 6 studies as opposed to the previous period where no publications were identified.

About 48 countries and 3 regions were identified in published studies on MSM (table 3). Countries that produced most MSM related studies were China, (73 studies, about 28%), followed by USA (28 studies, about 8.5%) and Brazil (14 studies, about 5.4%). These are the same three countries that also most published over the period 2003-2013, but now with China surpassing the USA in number of publications.

Table 2: FSW recruitment methods by study country

Population	Methods						Total	
	Convenience	Purposive	Snowball	Cluster	RDS	TLS	N	%
FSW								
Bangladesh				1			1	1.2
Botswana	1	1				1	3	3.7
Brazil					5		5	6.1
Burkina-Faso					2		2	2.4
Cameroon			1		1		2	2.4
Chile						1	1	1.2
China	6	1	2	4	3		16	19.5
Cote D'Ivoire					1		1	1.2
Gambia			1				1	1.2
India		1		1			2	2.4
Indonesia		2	3				5	6.1
Iran		1			1		2	2.4
Kenya			2		1		3	3.7
México						3	3	3.7
Montenegro			1				1	1.2
Mozambique					1		1	1.2
Myanmar						1	1	1.2
Namibia					1		1	1.2
Nepal				2			2	2.4
Nigeria		1	1				2	2.4
Papua New Guinea					2		2	2.4

Rwanda						1	1	1.2
South Africa			1		5		6	7.3
Swaziland					1		1	1.2
Tanzania						3	3	3.7
Uganda					2	2	4	4.9
Ukraine						1	1	1.2
Vietnam						1	1	1.2
Zimbabwe				1	5		6	7.3
India, Kenya, Mozambique, South Africa					1		1	1.2
Togo, Burkina-Faso					1		1	1.2
Total (N)	7	7	12	9	33	14	82	100
Total (%)	8.5	8.5	14.6	11.0	40.2	17.1	100	

Table 3: MSM recruitment methods by study country

Population	Methods							Total	
	Convenience	Purposive	Snowball	Cluster	Internet	RDS	TLS	N	%
MSM									
Argentina						1		1	0.4
Brazil					2	11	1	14	5.4
Burkina-Faso						4		4	1.5
Cambodja	1			4				5	1.9
Cameroon						2		2	0.8
Canada						10	1	11	4.2
Chile						2		2	0.8
China	10	3	16		15	22	7	73	28.2
Colombia						2		2	0.8
Cote D'Ivoire						2		2	0.8
Ecuador						1		1	0.4
France							1	1	0.4
Georgia						1		1	0.4
Great Britain	1				2			3	1.2
Guatemala						2		2	0.8
Guatemala							1	1	0.4
India					2	9		11	4.2
Indonesia		1						1	0.4
Ireland					2			2	0.8
Italy					1			1	0.4

Kenya						1		1	0.4
Lebanon						3		3	1.2
Lesoto						1		1	0.4
Malawi						2		2	0.8
Malaysia					1			1	0.4
Mali						3		3	1.2
México						5		5	1.9
Mongolia						1		1	0.4
Mozambique						2		2	0.8
Myanmar						5		5	1.9
Nigeria			1		1	8		10	3.9
Papua New Guinea						3		3	1.2
Peru	1				1	1		3	1.2
Russia						3		3	1.2
Rwanda						1		1	0.4
San Salvador						1		1	0.4
South Africa			1			6		7	2.7
South Korea							1	1	0.4
Swaziland						1		1	0.4
Sweden					2			2	0.8
Taiwan					1			1	0.4
Tanzania						7		7	2.7
Thailand	1	1			1			3	1.2
Togo			1			3		4	1.5
Uganda			1			2		3	1.2

USA					12	6	4	22	8.5
Vietnam	3		3	1	1	4		12	4.6
África						3		3	1.2
Africa, USA					1			1	0.4
Ásia					1			1	0.4
Europe					3	1		4	1.5
French Antilles, French Guiana			1					1	0.4
Togo, Burkina-Faso						1		1	0.4
Total (N)	17	5	24	5	49	143	16	259	100
Total (%)	6.6	1.9	9.3	1.9	18.9	55.2	6.2	100	

After the systematic review, we developed a method that aimed improving the accuracy of HIV prevalence estimates obtained when the TLS method is used to survey HRP.

TLS has been subject to several upgrades and suggestions to improve its estimates, namely on new ways to determine weights and the use of sampling weights (Karon & Wejnert, 2012; Leon et al., 2015; Sommen et al., 2018). However, the use of weights in TLS research is not consistent (Gustafson et al., 2013; Raymond et al., 2019) perhaps because it is difficult to identify the number of persons who are at the venue that belong to the population being studied or to know the number of times a person visits the venues (Lee, Wagner, Valliant, & Heeringa, 2014). In fact, several authors highlighted that the heterogeneity of the FVA can distort the accuracy and efficacy of TLS (Leon et al., 2015).

The underlying idea behind the creation of the method was to be simple to understand and easy to apply. Logistic regression is a statistical technique widely used in Health research, namely to analyse risk behaviours associated with HIV prevalence (Kleinbaum & Klein, 2010; Logie et al., 2017). To each observation, or to each respondent, logistic regression model gives residuals, which are the difference between the observed and the expected values (Zhang, 2016). These residuals can be determined in several ways with Pearson and Deviance methods being the most used. The method CARES, which means Calibration on Residuals, consists in imputing weights to the respondents, considering the percentile to which their residuals belong. The HIV prevalence is then estimated considering the CARES weights.

On the second paper, using a country database, a simulation was run to assess CARES performance and accuracy. The Spanish MSM sub-sample of the EMIS-2010 survey was chosen to test the method. The Spanish database, with 9591 men and an HIV prevalence of 12.1%, was considered the Spanish MSM HIV prevalence. About 35% of the population was eliminated from the database (either because did not report the region were they were living, because reported living in regions with less than 150 respondents or randomly selected) because TLS method tends to include venues where many persons congregate and only has access to persons who frequent them (Leon et al., 2015), this population was termed as population-base. The decision of excluding about 35% of the overall Spanish database was empirical because we did not find any literature referring to the coverage rate of a HRP by the TLS method. In fact, research on the population size estimation of venue-based methods is very limited (Verdery, Weir, Reynolds, Mulholland, & Edwards, 2019) and remains a challenge (Wesson, Handcock, McFarland, & Raymond, 2015).

After defining the population-base comprising 6185 men with an HIV prevalence of 11.74%, respondents were (artificially) divided into venues. Men of each Spanish region were assigned into a venue. The criteria to define the number of venues in each region was empirical and assuming that the number of venues should be directly proportional to the number of respondents on that region. The number of persons by venue was decided taking into account some of the UNAIDS guidelines that advise that, if there are several options, not to focus on venues with fewer than 15 potential respondents (WHO, 2010) because venues with more people the higher the chance of interviewing. On average, there were 90 men per venue, with the minimum of 34 and a maximum of 140 persons.

From the population-base, three pseudo-populations were then created all assuming an underestimation of the HIV prevalence:

- Pseudo-population 1 with 6057 individuals and an HIV prevalence of 9.87%;
- Pseudo-population 2 with 6105 individuals and an HIV prevalence of 10.58%;
- Pseudo-population 3 with 6162 individuals and an HIV prevalence of 11.41%.

From each pseudo-population two TLS simulation were run according to the following description:

1. On first stage 25% (n=17) of the venues were selected using a PPS approach and on second stage a response rate of 0.7 was considered; 1000 samples were drawn.
2. On first stage 20% (n=14) of the venues were selected using a PPS approach and on second stage a response rate of 0.7 was considered; 1000 samples were drawn.

For each sample in both simulations (1 and 2) weighted and unweighted estimated HIV prevalence was recorded, a logistic regression model was applied and Pearson and Deviance residuals were recorded.

CARES method was applied considering eight different scenarios:

a) Weight=1 to individuals whose residues are below the percentile 5 and weight=2 to individuals whose residues are equal or above the percentile 5;
b) Weight=1 to individuals whose residues are below the percentile 5 and weight=3 to individuals whose residues are equal or above the percentile 5;
c) Weight=1 to individuals whose residues are below the percentile 5.5 and weight=2 to individuals whose residues are equal or above the percentile 5.5;
d) Weight=1 to individuals whose residues are below the percentile 5.5 and weight=3 to individuals whose residues are equal or above the percentile 5.5;
e) Weight=1 to individuals whose residues are below the percentile 6 and weight=2 to individuals whose residues are equal or above the percentile 6;
f) Weight=1 to individuals whose residues are below the percentile 6 and weight=3 to individuals whose residues are equal or above the percentile 6;
g) Weight=1 to individuals whose residues are below the percentile 6.5 and weight=2 to individuals whose residues are equal or above the percentile 6.5;
h) Weight=1 to individuals whose residues are below the percentile 6.5 and weight=3 to individuals whose residues are equal or above the percentile 6.5;

For each of these eight scenarios the HIV estimated prevalence was again recorded.

HIV estimated results obtained with CARES were compared with the HIV prevalence estimated before the method CARES was applied (from now on called TLS HIV). Results showed that CARES improves the prevalence of TLS HIV estimates and that there were no significant differences when using Deviance or Pearson residuals. Simulations using the small sampling size (20% of the venues) provided similar HIV prevalence estimates but slightly wider confidence intervals when compared to simulations using 25% of the venues. When the HIV prevalence of the pseudo-population is very close to the (proxy) real HIV prevalence (i.e. less than 1% of difference, as in the case of pseudo-population 3), CARES overestimates results, therefore some caution must be taken when choosing the weights and the percentiles in order to avoid overestimate the results.

On the third study another simulation, similar to the previously described, was run to confirm CARES performance. Here we compared results obtained by simulation using the Spanish pseudo-population 1 and a Portuguese pseudo-population.

The Spanish pseudo-population 1 had 6057 men with an HIV prevalence of 9.9% distributed by 10 regions and 68 venues with an average of 89 men per venue, the smallest venue had 29 men and the largest venue had 166 men.

The Portuguese MSM sub-sample of the EMIS-2010 survey, with 3720 individuals and an HIV prevalence of 10.8%, was considered to be the (proxy) Portuguese MSM HIV prevalence. Similar to the Spanish case, about 15% of the population was eliminated from the database (either because did not report the region were they were living, because reported living in regions with less than 30 respondents or randomly selected) because TLS method only has access to persons who go to venues. The decision of excluding only about 15% of the overall database to approximate a TLS population was due to logistic regression size requirements. A pseudo-population was then created with 3160 individuals and an HIV prevalence of 9.2%. In each Portuguese region the pseudo-population was divided into venues. Similar to the Spanish case, the criteria to define the number of venues in each region was empirical and assuming that the number of venues should be directly proportional to the number of respondents in the region. Portuguese respondents were distributed by 12 regions and 44 venues with an average of 72 men per venue, the smallest venue had 34 men and the largest venue had 114 men.

From each pseudo-population (Portuguese and Spanish) TLS samples were simulated according to the following description:

1. On first stage 30% (n=20 Spanish, n=13 Portuguese) of the venues were selected using a PPS approach and on second stage a response rate of 0.7 was considered; 1000 samples were drawn.
2. On first stage 25% (n=17 Spanish, n=11 Portuguese) of the venues were selected using a PPS approach and on second stage a response rate of 0.7 was considered; 1000 samples were drawn.

In this third study we selected 30% and 25% of the venues and not 25% and 20% as in the second study because of the small size of the Portuguese database.

For each sample in both countries and simulations (1 and 2) weighted and unweighted estimated HIV prevalence was recorded, a logistic regression model was applied and Pearson and Deviance residuals were recorded.

CARES method was applied considering the same eight different scenarios previously described. HIV estimated results obtained with CARES were compared with the HIV prevalence estimated before the method CARES was applied (from now on called TLS HIV) for both countries.

Results showed that CARES improved TLS HIV estimates also in the Portuguese case. Results were similar for both sample sizes (30% and 25%), though wider confidence intervals and greater standard errors were produced for the small sample sizes. There were no significant differences when using Deviance or Pearson residuals.

Portuguese and Spanish MSM databases have different sizes and also different levels of HIV prevalence but results showed that CARES performed equally well on both cases.

In both simulation works, paper II and paper III, we presented the results of the estimated TLS HIV prevalence weighted and non-weighted by the sampling weights. Consequently, CARES method was applied to both weighted and non-weighted HIV estimates and results showed that CARES performed slightly better on weighted results for both countries and for both sample sizes.

Imputing weight equal to one to the lower percentiles and weight equal to two (or three) to the other percentiles, CARES gives less weight to those respondents with low risk behaviours and more weight to the remaining. As a consequence, HIV estimates increase and therefore, approximate to the (proxy) real HIV prevalence.

The decision to present the results for the percentiles 5, 5.5, 6, 6.5 and for the CARES weights=1, 2 and 1, 3 was made after running several tests. Given that the true prevalence of HIV in the population (in this case in the MSM population) is unknown, several scenarios (pseudo-populations with distinct HIV prevalences) were assumed and various weights applied to different percentiles of residues were tested in order to assess the behaviour of CARES estimator. Results obtained using the percentiles 5, 5.5, 6, 6.5

and applying the weights 1, 2 and 1, 3 provided the best estimates for all pseudo-populations tested.

This work is pioneer in using calibration weights based on the residuals of the logistic regression estimation as a method to improve the HIV prevalence estimates obtained when the time-location sampling method is used. In order to evaluate its feasibility we resort to the use of simulations to test several approaches and assess their performance. We also had to assume some premises such as the HIV prevalence being underestimated. CARES cannot yet be formally called a method but rather a starting point to a new post-estimation approach to be applied whenever the circumstances do not allow getting data with accuracy and precision.

International Health aims to identify, develop and implement practices and policies that help the world’s most vulnerable people improve their health and well-being (WHO, 2018) and in order to do it has a multidisciplinary and interdisciplinary approach. In this work we made use of statistical knowledge as a tool to improve the estimated results obtained for an international health problem, as it is the HIV infection on HRP.

Improving HIV prevalence estimates on HRP is an important step to better identify the reality and the needs of these populations, to better adequate tailored interventions such as the use of anti-retroviral therapy for prevention and therefore to improve their health and well-being.

3.2 Limitations

Limitations of this thesis were already identified in the papers. Most of them are related to the fact that the CARES method is a novel approach. Everything new is incipient and we are aware that this is a very simple analysis therefore it needs to be further developed. We acknowledge the following main limitations:

First, we are assuming that HIV is underestimated on HRP. Although several studies suggested it we cannot assure that it always happens.

Second, we excluded from the Spanish database about 35% of respondents and from the Portuguese database about 15% of respondents in order to approach the databases as close as possible to a TLS sampling frame. We are unaware if these numbers have some resemblance with reality.

Third, we only used sampling weights to calculate weighted prevalences but there are several other weighting techniques, such as the frequency of venue attendance that should be applied. Additionally, we assumed that all respondents had the same probability of inclusion in the samples, which might not be true.

Fourth, the PPS approach to sampling the venues and the assumption of a response rate of 70% within each cluster might be unreal.

Fifth, we selected seven risk factors to run the logistic regression analysis but we do not know what results would came up if other variables were used.

Sixth, imputing CARES weight=1 to men whose residuals belong to the first percentiles we are assuming that they have low risk behaviours however we cannot assure that this always happens, we did not look for possible outliers.

Seventh, we used the EMIS subsamples of Portugal and Spain to run the simulation that were collected through the Internet. Respondents' behaviour selected from Internet might be different from respondents selected through other sampling methods and therefore results might be biased.

Lastly, Portuguese and Spanish databases have some major similarities concerning socio and behavioural characteristics of the respondents. We wonder how CARES would have behaved if we tested the method on other databases with totally different characteristics.

3.3 Conclusions

This dissertation intended to contribute to lessen an International Health problem, as it is the HIV infection and its inaccurate estimates in hard-to-reach populations.

This thesis proposed a calibration method that could improve the accuracy of HIV prevalence estimates obtained when time-location sampling method is used to survey HRP.

TLS is a semi-probabilistic method that recruits a great number of respondents but its HIV prevalence estimates are prone to bias. The logistic regression estimates are often used in Health to assess the respondents' characteristics and risk behaviours.

The CARES method, which means calibration on residuals, assigns a specific weight to respondents considering the percentile to which their logistic regression residues belong.

The performance of CARES method was measured through the use of simulations made using real databases of HRP of MSM from Portugal and Spain. The method was tested assuming different levels of HIV prevalence, different sampling sizes and different weights.

Results showed that CARES method improves HIV prevalence estimates obtained through the use of time location sampling method.

We believe that CARES method has the potential to become a valuable International Health tool to be used when the populations' characteristics in study do not allow using more precise methods than those currently used, contributing in this way to ensure that all populations can benefit from adequate health care.

3.4 References

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ANNEX

Annex

List of publications of the systematic literature review update from 2014 to 2019, by population, method and country

Country	FSW METHODS
	CONVENIENCE
Botswana	Oduetse, O. K., Nkomo, B., Majingo, N., Mashalla, Y., & Seloilwe, E. (2019). Perceptions and attitudes towards acceptability of HIV self-testing among female sex workers in Selibe Phikwe, Botswana. *African Journal of AIDS Research : AJAR*, *18*(§), 192–197. https://doi.org/10.2989/16085906.2019.1638427
China	Dong, W., Zhou, C., Rou, K.-M., Wu, Z.-Y., Chen, J., Scott, S. R., … Chen, X. (2019). A community-based comprehensive intervention to reduce syphilis infection among low-fee female sex workers in China: a matched-pair, community-based randomized study. *Infectious Diseases of Poverty*, *8*(1), 97. https://doi.org/10.1186/s40249-019-0611-z
China	Han, L., Zhou, C., Li, Z., Poon, A. N., Rou, K., Fuller, S., … Bulterys, M. (2016). Differences in risk behaviours and HIV/STI prevalence between low-fee and medium-fee female sex workers in three provinces in China. *Sexually Transmitted Infections*, *92*(4), 309–315. https://doi.org/10.1136/sextrans-2015-052173

China	Zeng, H., Zhang, L., Zhao, Y., Liu, H., Guo, H., Wang, Y., … Mao, L. (2016). HIV prevention among street-based sex workers (SSWs) in Chongqing, China: interviews with SSWs, clients and healthcare providers. *Health & Social Care in the Community*, *24*(§), e173–e180. https://doi.org/10.1111/hsc.12266
China	Zhu, J., Hu, D., Yin, Y., Zhu, Z., Wang, N., & Wang, B. (2019). HIV prevalence and correlated factors among male clients of female sex workers in a border region of China. *PloS One*, *14*(§), e0225072–e0225072. https://doi.org/10.1371/journal.pone.0225072
China	Zhu, J., Lian, D. S., Yin, Y. Q., Hu, D., Wang, N., Chen, L. S., & Wang, B. (2019). HIV prevalence and correlated factors on male clients of female sex workers in Hekou Yao autonomous county of Yunnan province, 2014-2015. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi*, *40*(§), 440–445. https://doi.org/10.3760/cma.j.issn.0254-6450.2019.04.013
China	Zhu, J., Yuan, R., Hu, D., Zhu, Z. B., Yang, X., Wang, N., & Wang, B. (2017). Consecutive cross-sectional survey of prevalence of HIV infection/STD and related factors in Vietnamese female sex workers at a China-Vietnam border area, 2014-2015. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi*, *38*(5), 638–642. https://doi.org/10.3760/cma.j.issn.0254-6450.2017.05.016

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Botswana	Oduetse, O. K., Nkomo, B., Majingo, N., Mashalla, Y., & Seloilwe, E. (2019). Perceptions and attitudes towards acceptability of HIV self-testing among female sex workers in Selibe Phikwe, Botswana. *African Journal of AIDS Research : AJAR*, *18*(§), 192–197. https://doi.org/10.2989/16085906.2019.1638427
China	Zeng, H., Zhang, L., Zhao, Y., Liu, H., Guo, H., Wang, Y., … Mao, L. (2016). HIV prevention among street-based sex workers (SSWs) in Chongqing, China: interviews with SSWs, clients and healthcare providers. *Health & Social Care in the Community*, *24*(§), e173–e180. https://doi.org/10.1111/hsc.12266

India	Blanchard, A. K., Nair, S. G., Bruce, S. G., Sangha, C. A. T. M., Ramanaik, S., Thalinja, R., … Bhattacharjee, P. (2018). A community-based qualitative study on the experience and understandings of intimate partner violence and HIV vulnerability from the perspectives of female sex workers and male intimate partners in North Karnataka state, India. *BMC Women's Health*, *18*(§), 66. https://doi.org/10.1186/s12905-018-0554-8
Indonesia	Fauk, N. K., Kustanti, C. Y., Wulandari, R., Damayani, A. D., & Mwanri, L. (2018). Societal determinants of HIV vulnerability among clients of female commercial sex workers in Indonesia. *PloS One*, *13*(11), e0207647–e0207647. https://doi.org/10.1371/journal.pone.0207647
Indonesia	Fauk, N. K., Sukmawati, A. S., Berek, P. A. L., Ernawati, Kristanti, E., Wardojo, S. S. I., … Mwanri, L. (2018). Barriers to HIV testing among male clients of female sex workers in Indonesia. *International Journal for Equity in Health*, *17*(§), 68. https://doi.org/10.1186/s12939-018-0782-4
Iran	Asadi-Aliabadi, M., Abolghasemi, J., Rimaz, S., Majdzadeh, R., Rostami-Maskopaee, F., & Merghati-Khoei, E. (2018). Barriers to Health Service Utilization Among Iranian Female Sex Workers: A Qualitative Study. *Journal of Preventive Medicine and Public Health = Yebang Uihakhoe Chi*, *51*(§), 64–70. https://doi.org/10.3961/jpmph.17.174
Nigeria	Ilesanmi, R. E., & Kehinde, D. R. (2018). Pattern of Utilization of Cervical Cancer Screening Services among Female Sex Workers in Some Selected Brothels in Abuja, Nigeria. *Asia-Pacific Journal of Oncology Nursing*, *5*(4), 415–420. https://doi.org/10.4103/apjon.apjon_31_18
	Pinedo González, R., Palacios Picos, A., & de la Iglesia Gutiérrez, M. (2018). "Surviving the Violence, Humiliation, and Loneliness Means Getting High": Violence, Loneliness, and Health of Female Sex Workers. *Journal of Interpersonal Violence*, 886260518789904–886260518789904. https://doi.org/10.1177/0886260518789904

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Cameroon	Decker, M. R., Lyons, C., Billong, S. C., Njindam, I. M., Grosso, A., Nunez, G. T., … Baral, S. (2016). Gender-based violence against female sex workers in Cameroon: prevalence and associations with sexual HIV risk and access to health services and justice. *Sexually Transmitted Infections*, *92*(§), 599–604. https://doi.org/10.1136/sextrans-2015-052463
China	Yang, Y., Yang, C., Latkin, C. A., Luan, R., & Nelson, K. E. (2016). Condom Use During Commercial Sex Among Male Clients of Female Sex Workers in Sichuan China: A Social Cognitive Theory Analysis. *AIDS and Behavior*, *20*(§), 2309–2317. https://doi.org/10.1007/s10461-015-1239-z
China	Zhu, J., Hu, D., Yin, Y., Zhu, Z., Wang, N., & Wang, B. (2019). HIV prevalence and correlated factors among male clients of female sex workers in a border region of China. *PloS One*, *14*(§), e0225072–e0225072. https://doi.org/10.1371/journal.pone.0225072
Gambia	Grosso, A. L., Lei, E. L., Ketende, S. C., Peitzmeier, S., Mason, K., Ceesay, N., … Baral, S. (2015). Correlates of condom use among female sex workers in The Gambia: results of a cross-sectional survey. *PeerJ*, *3*, e1076–e1076. https://doi.org/10.7717/peerj.1076
Indonesia	Fauk, N. K., Kustanti, C. Y., Wulandari, R., Damayani, A. D., & Mwanri, L. (2018). Societal determinants of HIV vulnerability among clients of female commercial sex workers in Indonesia. *PloS One*, *13*(11), e0207647–e0207647. https://doi.org/10.1371/journal.pone.0207647
Indonesia	Fauk, N. K., Sukmawati, A. S., Berek, P. A. L., Ernawati, Kristanti, E., Wardojo, S. S. I., … Mwanri, L. (2018). Barriers to HIV testing among male clients of female sex workers in Indonesia. *International Journal for Equity in Health*, *17*(§), 68. https://doi.org/10.1186/s12939-018-0782-4
Indonesia	Mwanri, L., Fauk, N. K., Kustanti, C. Y., Ambarwati, A., & Merry, M. S. (2018). HIV susceptibility among clients of female sex workers in Indonesia: a qualitative inquiry. *Sexual Health*, *15*(§), 246–253. https://doi.org/10.1071/SH17137

Kenya	Menon, S., Broeck, D. Vanden, Rossi, R., Ogbe, E., Harmon, S., & Mabeya, H. (2016). Associations Between Vaginal Infections and Potential High-risk and High-risk Human Papillomavirus Genotypes in Female Sex Workers in Western Kenya. *Clinical Therapeutics*, *38*(12), 2567–2577. https://doi.org/10.1016/j.clinthera.2016.10.005
Kenya	Nyblade, L., Reddy, A., Mbote, D., Kraemer, J., Stockton, M., Kemunto, C., … Barker, C. (2017). The relationship between health worker stigma and uptake of HIV counseling and testing and utilization of non-HIV health services: the experience of male and female sex workers in Kenya. *AIDS Care*, *29*(§), 1364–1372. https://doi.org/10.1080/09540121.2017.1307922
Montenegro	Laušević, D., Begić, S., Terzić, N., Vratnica, Z., Čičić, A., & Mugoša, B. (2016). Factors associated with inconsistent condom use with clients among female sex workers in Podgorica, Montenegro. *Srpski Arhiv Za Celokupno Lekarstvo*, *144*(7–8), 424–430. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29652452
Nigeria	Nelson, E.-U. E. (2019). The lived experience of violence and health-related risks among street sex workers in Uyo, Nigeria. *Culture, Health & Sexuality*, 1–14. https://doi.org/10.1080/13691058.2019.1648872
South Africa	Mukumbang, F. C. (2017). Actions of female sex workers who experience male condom failure during penetrative sexual encounters with clients in Cape Town: Implications for HIV prevention strategies. *Southern African Journal of HIV Medicine*, *18*(§), 698. https://doi.org/10.4102/sajhivmed.v18i1.698

CLUSTER

Bangladesh	Katz, K. R., McDowell, M., Green, M., Jahan, S., Johnson, L., & Chen, M. (2015). Understanding the Broader Sexual and Reproductive Health Needs of Female Sex Workers in Dhaka, Bangladesh. *International Perspectives on Sexual and Reproductive Health*, *41*(4), 182–190. https://doi.org/10.1363/4118215

China	Lai, J., Qin, C., Nehl, E. J., Jiang, J., Huang, Y., Liang, B., … Liang, H. (2018). HIV prevalence among female sex workers in Guigang City, Guangxi, China: an 8-year consecutive cross-sectional study. *BMC Public Health*, *18*(1), 450. https://doi.org/10.1186/s12889-018-5380-2
China	Lim, M. S. C., Zhang, X.-D., Kennedy, E., Li, Y., Yang, Y., Li, L., … Luchters, S. (2015). Sexual and reproductive health knowledge, contraception uptake, and factors associated with unmet need for modern contraception among adolescent female sex workers in China. *PloS One*, *10*(1), e0115435–e0115435. https://doi.org/10.1371/journal.pone.0115435
China	Liu, M., Xiao, C. C., Wang, T., Liu, J. F., Li, S. Y., & Yan, H. (2016). Association between HIV infection and female sex workers in middle aged and old men in a city in Hubei provine, 2014. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi*, *37*(§), 965–969. https://doi.org/10.3760/cma.j.issn.0254-6450.2016.07.012
China	Wang, J., Lin, F., Yang, Y., Yu, F., Zhao, Y., He, N., & Zhang, T. (2015). Research on the smoking status and influence factors among female sex workers and general migrant women in Shanghai City. *Wei sheng yan jiu = Journal of hygiene research*, *44*(2), 206–210. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25997220
India	Prakash, R., Manthri, S., Tayyaba, S., Joy, A., Raj, S. S., Singh, D., & Agarwal, A. (2016). Effect of Physical Violence on Sexually Transmitted Infections and Treatment Seeking Behaviour among Female Sex Workers in Thane District, Maharashtra, India. *PloS One*, *11*(3), e0150347–e0150347. https://doi.org/10.1371/journal.pone.0150347
Nepal	Deuba, K., Anderson, S., Ekström, A. M., Pandey, S. R., Shrestha, R., Karki, D. K., & Marrone, G. (2016). Micro-level social and structural factors act synergistically to increase HIV risk among Nepalese female sex workers. *International Journal of Infectious Diseases : IJID : Official Publication of the International Society for Infectious Diseases*, *49*, 100–106. https://doi.org/10.1016/j.ijid.2016.06.007
Nepal	Kakchapati, S., Paudel, T., Maharjan, M., & Lim, A. (2016). Systematic Differences in HIV, Syphilis and Risk Behaviors among Street Based and Establishment Based Female Sex Workers in Kathmandu Valley of Nepal. *Nepal Journal of Epidemiology*, *6*(4), 620–630. https://doi.org/10.3126/nje.v6i4.17256

Zimbabwe	Cowan, F. M., Davey, C., Fearon, E., Mushati, P., Dirawo, J., Chabata, S., … Hargreaves, J. R. (2018). Targeted combination prevention to support female sex workers in Zimbabwe accessing and adhering to antiretrovirals for treatment and prevention of HIV (SAPPH-IRe): a cluster-randomised trial. *The Lancet. HIV*, *5*(8), e417–e426. https://doi.org/10.1016/S2352-3018(18)30111-5

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Brazil	Damacena, G. N., Szwarcwald, C. L., Souza Júnior, P. R. B. de, Ferreira Júnior, O. da C., Almeida, W. da S. de, Pascom, A. R. P., … Group, B. F. S. W. (2019). Application of the Respondent-Driven Sampling methodology in a biological and behavioral surveillance survey among female sex workers, Brazil, 2016. *Revista Brasileira de Epidemiologia = Brazilian Journal of Epidemiology*, *22Suppl 1*(Suppl 1), e190002–e190002. https://doi.org/10.1590/1980-549720190002.supl.1
Brazil	Ferreira-Júnior, O. da C., Guimarães, M. D. C., Damacena, G. N., de Almeida, W. da S., de Souza-Júnior, P. R. B., Szwarcwald, C. L., & Group, B. F. S. W. (2018). Prevalence estimates of HIV, syphilis, hepatitis B and C among female sex workers (FSW) in Brazil, 2016. *Medicine*, *97*(1S Suppl 1), S3–S8. https://doi.org/10.1097/MD.0000000000009218
Brazil	Szwarcwald, C. L., Damacena, G. N., de Souza-Júnior, P. R. B., Guimarães, M. D. C., de Almeida, W. da S., de Souza Ferreira, A. P., … Group, B. F. S. W. (2018). Factors associated with HIV infection among female sex workers in Brazil. *Medicine*, *97*(1S Suppl 1), S54–S61. https://doi.org/10.1097/MD.0000000000009013
Brazil	Szwarcwald, C. L., de Almeida, W. da S., Damacena, G. N., de Souza-Júnior, P. R. B., Ferreira-Júnior, O. da C., Guimarães, M. D. C., & Group, B. F. S. W. (2018). Changes in attitudes, risky practices, and HIV and syphilis prevalence among female sex workers in Brazil from 2009 to 2016. *Medicine*, *97*(1S Suppl 1), S46–S53. https://doi.org/10.1097/MD.0000000000009227

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Cote D'Ivoire	Lyons, C. E., Grosso, A., Drame, F. M., Ketende, S., Diouf, D., Ba, I., … Baral, S. (2017). Physical and Sexual Violence Affecting Female Sex Workers in Abidjan, Côte d'Ivoire: Prevalence, and the Relationship with the Work Environment, HIV, and Access to Health Services. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *75*(1), 9–17. https://doi.org/10.1097/QAI.0000000000001310

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Zimbabwe	Cowan, F. M., Davey, C. B., Fearon, E., Mushati, P., Dirawo, J., Cambiano, V., … Hargreaves, J. R. (2017). The HIV Care Cascade Among Female Sex Workers in Zimbabwe: Results of a Population-Based Survey From the Sisters Antiretroviral Therapy Programme for Prevention of HIV, an Integrated Response (SAPPH-IRe) Trial. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *74*(4), 375–382. https://doi.org/10.1097/QAI.0000000000001255
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Uganda	Goldenberg, S. M., Muzaaya, G., Akello, M., Braschel, M., Birungi, J., & Shannon, K. (2019). High burden of previously undiagnosed HIV infections and gaps in HIV care cascade for conflict-affected female sex workers in northern Uganda. *International Journal of STD & AIDS, 30*(3), 275–283. https://doi.org/10.1177/0956462418804658

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Ukraine	Tokar, A., Sazonova, I., Mishra, S., Smyrnov, P., Saliuk, T., Lazarus, J. V, … Becker, M. L. (2019). HIV testing behaviour and HIV prevalence among female sex workers in Ukraine: findings from an Integrated Bio-Behavioural Survey, 2013-2014. *Sexually Transmitted Infections*, *95*(3), 193–200. https://doi.org/10.1136/sextrans-2018-053684
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Country	MSM METHODS
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China	Cai, Y. M., Song, Y. J., Liu, H., & Hong, F. C. (2016). Factors associated with commercial sexual behavior among men who have sex with men in Shenzhen, China, in 2011-2015. *Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]*, *50*(11), 943–948. https://doi.org/10.3760/cma.j.issn.0253-9624.2016.11.005
China	Dai, W., Luo, Z., Xu, R., Zhao, G., Tu, D., Yang, L., … Feng, T. (2017). Prevalence of HIV and syphilis co-infection and associated factors among non-commercial men who have sex with men attending a sexually transmitted disease clinic in Shenzhen, China. *BMC Infectious Diseases*, *17*(1), 86. https://doi.org/10.1186/s12879-017-2187-1
China	Fu, G.-F., Jiang, N., Hu, H.-Y., Mahapatra, T., Yin, Y.-P., Mahapatra, S., … Tang, W. (2015). The epidemic of HIV, syphilis, chlamydia and gonorrhea and the correlates of sexual transmitted infections among men who have sex with men in Jiangsu, China, 2009. *PloS One*, *10*(3), e0118863–e0118863. https://doi.org/10.1371/journal.pone.0118863

China	Hao, Y. X., Qin, Q. Q., Wu, G. H., Zhang, W., Guo, W., Cui, Y., … Sun, J. P. (2017). Analysis of Spatial Clustering of HIV infected in men who have sex with men in Chongqing of 2004-2015. *Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]*, *51*(5), 432–437. https://doi.org/10.3760/cma.j.issn.0253-9624.2017.05.012
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China	Wu, D., Tao, H., Dai, J., Liang, H., Huang, A., & Zhong, X. (2019). Study on Pre-Exposure Prophylaxis Regimens among Men Who Have Sex with Men: A Prospective Cohort Study. *International Journal of Environmental Research and Public Health*, *16*(24), E4996. https://doi.org/10.3390/ijerph16244996
China	Xu, R., Dai, W., Zhao, G., Tu, D., Yang, L., Wang, F., … Kaminga, A. C. (2016). Early Sexual Debut and HIV Infection among Men Who Have Sex with Men in Shenzhen, China. *BioMed Research International*, *2016*, 2987472. https://doi.org/10.1155/2016/2987472
China	Zeng, X., Zhong, X., Peng, B., Zhang, Y., Kong, C., Liu, X., & Huang, A. (2016). Prevalence and associated risk characteristics of HIV infection based on anal sexual role among men who have sex with men: a multi-city cross-sectional study in Western China. *International Journal of Infectious Diseases : IJID : Official Publication of the International Society for Infectious Diseases*, *49*, 111–118. https://doi.org/10.1016/j.ijid.2016.06.009
China	Zhang, X. F., Lyu, C. X., Zheng, J. T., Dong, L., Jiang, Z. X., & Fu, J. H. (2017). Status on sexual partners among men who have sex with men among college students in Qingdao, 2016. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi*, *38*(12), 1638–1641. https://doi.org/10.3760/cma.j.issn.0254-6450.2017.12.011

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Vietnam	Hershow, R. B., Miller, W. C., Giang, L. M., Sripaipan, T., Bhadra, M., Nguyen, S. M., … Go, V. F. (2018). Minority Stress and Experience of Sexual Violence Among Men Who Have Sex With Men in Hanoi, Vietnam: Results From a Cross-Sectional Study. *Journal of Interpersonal Violence,* 886260518819884–886260518819884. https://doi.org/10.1177/0886260518819884
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