Sexual behaviors and risk of extended-spectrum β-lactamase-producing Enterobacterales carriage: A cross-sectional analysis

Anders Boyd, Pauline Mathieu, Ugo Françoise, Hayette Rougier, Thibault Chiarabini, Nadia Valin, Karine Lacombe, Paul-Louis Woerther, Laure Surgers

Sexual behaviors and risk of extended-spectrum β-lactamase-producing Enterobacterales carriage: a cross-sectional analysis

Anders Boyd[1,2,3], Pauline Mathieu[1], Ugo Françoise[4], Hayette Rougier[5], Thibault Chiarabini[4], Nadia Valin[4], Karine Lacombe[1,4], Paul-Louis Woerther[6,7], Laure Surgers[1,4]

Institutional affiliations:

[1]Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie et de Santé Publique, Paris, France;

[2]Stichting hiv monitoring, Amsterdam, the Netherlands;

[3]Department of Infectious Diseases, Research and Prevention, Public Health Service of Amsterdam, Amsterdam, the Netherlands;

[4]GHU AP-HP, Sorbonne Université, Service des Maladies Infectieuses et Tropicales, Hôpital Saint-Antoine, Paris, France;

[5]Institut de Médecine et d'Epidémiologie Appliquée, Paris, France;

[6]Unité de Bactériologie, Département de Prévention, Diagnostic et Traitement des Infections, CHU Henri Mondor, AP-HP;

[7]EA DYNAMiC 7380, Faculté de Santé, Univ Paris-Est Créteil, 94000 Créteil, France.

Corresponding Author:

Dr. Laure Surgers, MD, PhD

Service des Maladies Infectieuses et Tropicales,

AP-HP Hôpital Saint-Antoine

184 rue du Faubourg Saint-Antoine

75012 Paris, France

E-mail: laure.surgers@aphp.fr

ABSTRACT

Objectives: Sexual transmission of extended-spectrum β-lactamase-producing Enterobacterales (ESBL-E) is suspected. We aimed to identify clusters of sexual behavior associated with ESBL-E carriage among individuals at risk of sexually transmitted infections (STI).

Methods: In this cross-sectional study, patients attending an STI-screening center and HIV outpatient clinic in Paris, France between 2018-2019 were asked questions on the following sexual activities in the last 6 months: receptive/insertive anal intercourse, passive/active rimming, receptive/insertive fellatio, receptive/insertive fisting, receptive/insertive fingering, active/passive cunnilingus. ESBL-E carriage was determined from rectal swabs. Bayesian latent class analysis was used to identify clusters of sexual activity, which were then associated with ESBL-E carriage using logistic regression.

Results: Among 1211 men who have sex with men (MSM), those belonging to two latent classes with higher prevalence of insertive fingering and active rimming (ESBL-E prevalence=15.3%, *N*=124 and 16.0%, *N*=100) and one class with higher proportions of all behaviors (24.3%, *N*=70) had a higher risk of ESBL-E carriage compared to those in a class with few sexual behaviors (7.3%, *N*=259) after adjustment. Among 439 other men and 479 women, no clear associations between sexual clusters and ESBL-E carriage were observed.

Conclusions: Sexual behaviors are associated with varying degrees of ESBL-E carriage, particularly among MSM.

KEYWORDS: antimicrobial resistance; Enterobacterales; sexually transmitted diseases; transmission; epidemiology.

INTRODUCTION

Enterobacterales is a large order of Gram-negative bacillus, found as commensal organisms in the digestive tract; and can lead to urinary, gastroenterological, and pulmonary infections. Of the Enterobacterales, the production of extended-spectrum β-lactamase (ESBL) is the principal mechanism of resistance to 3^{rd} generation cephalosporin antibiotics. The therapeutic options for individuals infected with these microorganisms are limited and almost always require the use of carbapenems. *Escherichia coli* is the most common ESBL-producing Enterbacterales (ESBL-E) in community [1]. The clinical progression of individuals with ESBL-E infection is more severe compared to those without, and includes longer hospital stays and increased rates of morbidity and mortality due to infection [2,3]. Carriage of ESBL-E is known to be a risk factor for subsequent infection [4,5].

Of concern, the prevalence of ESBL-E has been increasing in community settings, with a recent meta-analysis estimating an overall 10-fold increase in individuals colonized with ESBL-E between 2001 and 2020 [6]. In France, one study in a general healthcare center has observed an almost 10-fold increase in ESBL-E carriage, from 0.6% in 2006 to 6% in 2011 [7]. There are a wide variety of risk factors for ESBL-E carriage described in the literature: recent antibiotic therapy [8,9], hospitalization [10], travel to a region of high EBSL-E prevalence [11], and living in communal environments [12]. Nevertheless, information on other potential risk factors for developing ESBL-E carriage have been lacking and in 2010, the French High Counsel for Public Health (*Haut Conseil pour la Santé Publique*) recommended conducting studies to further understand other factors contributing to the dissemination of ESBL-E [13].

One putative mode of transmission of ESBL-E could be through sexual contact. Sexual transmission of other pathogens has been observed via the fecal-oral route, such as *Shigella* spp. [14,15] (and more recently *Shigella sonnei* carrying the blaCTX-M-27-producing plasmid [16]), hepatitis A virus [17], and *Campylobacter coli* [18,19]. Indeed, a recent study conducted at a sexual health center in Paris, France observed a high percentage of ESBL-E carriage, at roughly 15%, among men who have sex with men (MSM), particularly those on pre-exposure prophylaxis for human immunodeficiency virus (HIV) or living with HIV, when compared to other men or women [20]. This difference was mostly explained by increased numbers of recent sexual partners, which was associated with a higher probability of ESBL-E carriage.

These findings, coupled with others [21], illustrate that transmission of ESBL-E could be possible though close contact during sex. However, it is unclear which sexual behaviors could increase the risk of ESBL-E carriage. The aim of this study was then to examine the differences in sexual behaviors between individuals with and without ESBL-E carriage at a larger sexual healthcare clinic in Paris, France. Given the strong collinearity of sexual behaviors, we intended to study the clusters of sexual behaviors that could be driving ESBL-E carriage.

METHODS

Study design

We analyzed cross-sectional data from the *Bactéries MultiRésistantes-Infections sexuellement transmissibles* (BMR-IST) study [20]. In brief, this study is a two-part, mono-centric study conducted at Sorbonne Université Saint-Antoine Hospital (Assistance Publique-Hôpitaux de Paris, Paris, France) between 2018 and 2019. The first part was a cross-sectional assessment of the prevalence and risk-factors for carriage of ESBL-E, Vancomycin Resistant *Enterococci* (VRE) and Carbapenemase-Producing Enterobacterales (CPE) in various groups at risk of sexually transmitted infections (STI). This analysis includes only data from the first part of this cohort.

We included individuals who were ≥18 years, HIV-negative (seeking care at the Sexual Health Clinic) or HIV-positive (seeking care at the Department of Infectious and Tropical Diseases), and signed informed consent. We did not include HIV-positive men who exclusively have sex with women (MSW) or HIV-positive women who have sex with men (WSM). We also did not include individuals who were unable to proficiently read or speak French.

Study procedures

We asked individuals during regular outpatient consultations whether they would be interested in participating in the study. After obtaining written informed consent, we asked participants to fill out a questionnaire on demographic characteristics, ESBL-E risk factors and sexual behaviour with the assistance of a study investigator. The choice of STI tests was at the physician's discretion. We gave participants a self-swab (Copan ESwab™) and asked them to collect a sample in the lavatory of the clinic by inserting the swab into the rectal canal. Only swabs with visible fecal material were included in bacteriological analysis. After specimen collection, we sent samples to the Microbiology Department of Saint-Antoine Hospital, where they were tested for ESBL-E and stored at -80° C. All procedures relating to bacterial analysis have been described elsewhere in detail [20,22]. Detection of ESBL-E is described in the Supplementary materials.

Study variables

Demographic variables included age, geographic origin and whether participants were born in

France. Communal living was defined as living in a hotel, camp, or shelter, or being homeless.

Hospitalization within the past 12 months was recorded. We asked participants to list all antibiotics used in the last 6 months, including initiation and discontinuation dates, and all countries traveled to outside of France in the last 12 months, as well as entry and exit dates (or duration of travel) for each country. We regrouped countries according to region and ESBL-E country prevalence: low (<10%), medium (10-25%), and high (>25%) [11].

Gender was determined by the following categories: male, female, transgender male, transgender female, other. Variables on sexual activity in the past 6 months included any stable or known casual partners and any unknown casual partners, as well as questions on the gender and number of partners. We categorized the number of partners as 0-7, 8-30, or >30. We asked additional questions on whether a participant engaged in one of the following sexual behaviors in the past 6 months: insertive and receptive vaginal sex, insertive and receptive anal sex, insertive and receptive fellatio, active and passive cunnilingus, active and passive rimming, receptive and insertive fingering, and active or passive fisting. For each behavior, we asked if a condom was always, sometimes or never used. We also asked whether the participant was taking preexposure prophylaxis (PrEP) for HIV.

Statistical analysis

From questions on sexual activity, we characterized three sexual groups: MSM, other men (*i.e.*, men who reported having sex with exclusively women), and women. The limited numbers of transgender persons and those with other genders precluded further analysis specific to these individuals. Participant characteristics were summarized as count (%) for categorical and median [interquartile range (IQR)] for continuous variables according to sexual groups. These characteristics were compared between sexual groups using Pearson's χ^2 or Fisher's Exact test for categorical variables and Kruskal-Wallis test for continuous variables.

To determine which individual sexual behaviors were associated ESBL-E carriage, we modeled the probability of ESBL-E carriage using logistic regression. We included covariates in univariable analysis, from which the odds ratio (OR) and 95% confidence interval (CI), comparing the odds of ESBL-E carriage across levels of covariates, were calculated. We constructed a multivariable model to obtain adjusted ORs, which were adjusted *a priori* on other risk factors related to ESBL-E carriage (i.e., antibiotic use in the previous 6 months, highest ESBL-E prevalence of region travelled in the previous 6 months, and number of sexual partners in the previous 6 months). These models were carried out separately according to sexual group.

Given the highly correlated nature of sexual behaviors, we conducted a Bayesian latent class analysis (LCA) to construct classes (or clusters) of behaviors. Briefly, a set of binomially-distributed variables can be regrouped in to classes of participants with similar multivariate distributions. This can be carried out using a finite mixture model framework with expectation-maximization and uniform priors. We ran these models for each sexual group, separately, and the variables used to construct latent classes differed between these groups. The model for MSM included: receptive or insertive anal intercourse, passive or active rimming, receptive or insertive fellatio, receptive or insertive fisting, and receptive or insertive fingering. The model for other men included: insertive anal sex, passive or active rimming, active cunnilingus, receptive fellatio, and receptive or insertive fingering. The model for women included: receptive anal sex, receptive or insertive rimming, receptive cunnilingus, insertive fellatio, and receptive or insertive fingering.

For each sexual group, we ran model with sequentially increasing numbers of classes, *k*. We compared the fits between models using the Bayesian Information Criterion (BIC) and the model with the BIC closest to 0 was selected as the final model. From the likelihood of this model, we can calculate the posterior probability of belonging to each class k for individual i, π_{ik}. Each individual can then be assigned to a class of sexual behavior for which their posterior probability is the highest. The Bayesian LCA employed an expectation-maximization algorithm, which was performed with the "BayesLCA" package in R [23]. We performed additional diagnostic plots to assess the classification certainty of class membership across combinations of behaviors.

To order classes in a more clinically meaningful way, we computed the proportion of total sexual behaviors reported for each individual and calculated the mean of this proportion within clusters (*i.e*., "mean proportion of total behaviors"). The classes were then ordered from lowest to highest mean proportion of total behaviors. We modeled the probability of ESBL-E carriage using logistic regression and included class number as a covariate. We calculated the OR and 95%CI comparing the odds of carriage compared to the class with the lowest mean proportion of total behaviors. OR were presented in both univariable (unadjusted) and multivariable analysis (adjusted on antibiotic use in the previous 6 months and highest ESBL-E prevalence of region travelled in the previous 6 months).

Data management was performed using STATA (v15.1, College Station, TX, USA) and analysis carried out using R (version 4.0.3, Vienna, Austria). A p-value <0.05 was considered statistically significant. Statistical code for these analyses can be found on a public repository (https://github.com/boyd0094/BMRIST_sex_clusters).

RESULTS

Description of the study population

From 11 May 2018 to 30 June 2019, 2186 participants completed the questionnaire. 57 participants were excluded for the following reasons: no sample, *n*=12; did not consent for use of data, *n*=2; no sexual partners in the last 6 months, *n*=1; transgender woman or man *n*=14.did not answer any questions on specific sexual behavior, *n*=28. In total, 2129 were included in analysis. Of them, 1211 were MSM, 439 were other men, and 479 were women.

Characteristics of the study population are described and compared between sexual groups in Table 1. Compared to MSM, other men and women were younger (p<0.001), more likely to be in communal living (p=0.036), and less likely to have used antibiotics in the past 6 months (p<0.001). Other men and women also had a lower median number of sexual partners in the past 6 months (p<0.001) compared to MSM. In the 6 months before the study visit, MSM used PrEP (p<0.001), used drugs, and (p<0.001) had an STI (p<0.001) more often versus other men or women. Among the 1211 MSM, 489 (40.4%) were HIV-positive and of the 722 MSM without HIV, 246 (34.1%) were using PrEP.

The proportion of individuals engaging in the applicable sexual behaviors are described and compared between sexual groups in Table 2. There were significant differences between groups, where comparisons were possible, in all behaviors. Given the differences in study population characteristics and sexual behaviors, we stratified analysis on sexual group.

Association of individual sexual behaviors and ESBL-E carriage

The prevalence of ESBL-E carriage was highest among MSM (12.3%, 95%CI=10.5-14.3), followed by other men (10.0%, 95%CI=7.4-13.3) and women (6.9%, 95%CI=4.9-9.6) (p=0.0044). Of those with ESBL-E carriage, the most frequent ST types were ST131 (n=60, 26.5%), followed by ST14 (n=26, 11.5%) and ST1193 (n=22, 9.7%), while almost all ST14 and ST1193 strains were found among MSM. The majority of ESBL-E were CTX-M-15-producing (n=132, 58.4%), followed by CTX-M-27 (n=41, 18.1%) and SHV-12 (n=28, 12.4%). All SHV-12-producing ESBL-E strains were found in MSM.

The unadjusted and adjusted OR and 95%CI between each applicable sexual behavior and ESBL-E carriage are given across sexual groups in Table 3. In MSM, the following sexual behaviors were associated with a higher odds of ESBL-E carriage: receptive anal intercourse (p=0.034), receptive fisting (p=0.002) and insertive fisting (p=0.047). In other men, only receptive fellatio was associated with a lower odds of ESBL-E carriage (p=0.030). In women, no sexual behaviors were associated with ESBL-E carriage. All behaviors associated with ESBL-E carriage remained significant after adjustment, except for receptive anal intercourse and insertive fisting for MSM.

Association of latent classes of sexual behaviors and ESBL-E carriage

In MSM, the LCA model with the lowest BIC (Supplementary Table 1) revealed 7 classes of sexual behaviors, which are described in Figure 1A. The model produced high classification certainty across combinations of behaviors (Supplementary Figure S1A). As shown in Table 4, the mean proportion of total behaviors ranged from 29.9% in class 1 to 91.7% in class 7, while at the same time the prevalence of ESBL-E carriage ranged from 7.3% in class 1 to 24.3% in class 7. Characteristics of the study population are compared across these classes of MSM in Supplementary Table S2.

When compared to class 1, class 3 (including higher proportions of individuals with insertive fingering, insertive anal intercourse and active rimming), class 4 (including higher proportions of individuals with insertive fingering and active rimming), and class 7 (including higher proportions of individuals with all sexual behaviors) had significantly higher probability of ESBL-E carriage. These differences remained after adjustment for other risk factors related to ESBL-E carriage. Class 6 (including high proportions of individuals with all sexual behaviors except for insertive and receptive fisting, and was the only class in which participants reported fisting) also had a high prevalence of ESBL-E carriage compared to class 1 (p=0.047), but this was no longer significant after adjustment.

In other men, the LCA model with the lowest BIC (Supplementary Table 1) revealed 3 classes of sexual behaviors, which are described in Figure 1B. The model produced high classification certainty across combinations of behaviors (Supplementary Figure S1B). As shown in Table 4, the mean proportion of total behaviors ranged from 11.1% in class 1 to 79.0% in class 3, while the prevalence of ESBL-E carriage was 16.7%, 8.1%, and 11.4% for classes 1, 2, and 3, respectively. Characteristics of the study population are compared across these classes of other men in Supplementary Table S3. Compared to class 1, class 2 (including high proportions of individuals with insertive fingering, active cunnilingus and receptive fellatio) had a significantly lower prevalence of ESBL-E carriage, even after adjustment.

In women, the LCA model with the lowest BIC (Supplementary Table 1) revealed 3 classes of sexual behaviors, which are described in Figure 1C. The model produced high classification certainty across combinations of behaviors (Supplementary Figure S1C). As shown in Table 4, the mean proportion of total behaviors ranged from 7.6% in class 1 to 81.2% in class 3, while the prevalence of ESBL-E carriage was 9.4%, 7.0%, and 4.2% for classes 1, 2, and 3, respectively. Characteristics of the study population are compared across these classes of women in Supplementary Table S4. Compared to class 1, there were no classes of sexual behavior that were associated with ESBL-E carriage, also after adjustment.

DISCUSSION

In our study of a population of individuals at risk of acquiring STIs, we observed specific clusters of sexual behaviors that were associated with a higher prevalence of ESBL-E carriage in MSM, but not in other men or women. MSM belonging to the clusters that generally included insertive fingering and active rimming had higher prevalence of ESBL-E carriage compared to the class with few behaviors reported. In particular, MSM belonging to the group reporting almost all sexual behaviors included in the questionnaire had a prevalence of ESBL-E carriage above 24%, which is almost four times higher than estimates in the general population in Paris, dating from 2011 [7]. This study provides further insight into the role of sexual behaviors on ESBL-E dissemination.

A handful of studies have highlighted the high prevalence of ESBL-E carriage among MSM, particularly at risk of STIs [20,21]. For instance, one study conducted in Amsterdam, the Netherlands, showed a prevalence of ESBL-E carriage at 16.3%, which corresponds to 2-times higher than that of the general Dutch population [24]. Epidemics related to infections from Enterobacterales, such as *Shigella* and *E. coli*, have also been identified in MSM [25,26]. Coupled with the phylogenetic evidence that sexual behaviors are likely related to acquiring ESBL-E [27,28], these findings suggest the potential for onward transmission in this key population. However, very few of these studies have examined how sexual behaviors were linked to either carriage or infection.

Emerging data have illustrated the association between increased numbers of recent sexual partners and higher ESBL-E prevalence [20,21]. The LCA presented in this study allows to understand specific groups of sexual behaviors related to ESBL-E carriage. In MSM, there is a wide variability of concomitant sexual behaviors, as evidenced by the seven clusters of behaviors, with the prevalence of ESBL-E carriage ranging from 7.3-24.3% across clusters. Of note, the cluster with fisting, combined with other behaviors, had the highest prevalence. A previous study in Amsterdam, which also used LCA, demonstrated that rimming and fisting, combined with other sexual activities, was also associated with higher prevalence of ESBL-E carriage [21]. Furthermore, phylogenetic data from these individuals would suggest putative transmission clusters within sexual partners [28]. All these associations were independent of risk factors commonly associated with ESBL-E carriage.

It is nevertheless difficult to pinpoint which transmission mechanism is responsible for the increased risk of Enterobacterales [29], as sexual behaviors commonly co-occur. If we simply model each behavior, only receptive fisting emerges as a determinant of ESBL-E carriage in MSM. Simplistically, this result can only infer on the risk of ESBL-E carriage for that individual behavior. However, LCA allows classes of co-occurring sexual behaviors to be constructed, which can be used to model the probability of ESBL-E carriage. As such, the results from LCA can infer on the risk of ESBL-E for the *ensemble* of behaviors within a class. This is not to say the risk of ESBL-E carriage associated with receptive anal fisting was absent in LCA – rather receptive anal fisting commonly occurred within many sexual behaviors, which together gave rise to the highest probability of ESBL-E carriage. Given the composition of classes, it is conceivable that ESBL-E was acquired through sexual behaviors more involved with the gastrointestinal tract, *i.e.*, fecal-oral transmission.

It should be noted that MSM are not homogenous in their sexual behaviors and subsequently, risk of STIs. One large study conducted in the Netherlands among approximately 13,000 MSM demonstrated that the risk of STIs was highest in MSM who participate in group sex, chemsex and increased numbers of sexual partners [30]. Accordingly, certain clusters could have a higher prevalence of STIs, which then needed to be treated with antibiotics. The more frequent exposure to antibiotic therapy would have likely induced more selective pressures for ESBL-E to develop and thus higher prevalence of ESBL-E carriage. Nevertheless, we adjusted comparisons on antibiotic exposure and differences remained significant.

In other men and women, LCA was unable to differentiate any cluster of sexual behavior associated with higher ESBL-E prevalence, apart from one class bearing a lower risk of ESBL-E prevalence in other men. These analyses do have several caveats. The variables on sexual behaviors pertain to engaging in any one behavior during the past six months. As we did not ask questions on the frequency of each behavior, it is not possible to determine how often these behaviors needed to occur in order to attain a higher risk of ESBL-E carriage. From our previous analysis of the same cohort, MSM have many more sexual partners compared to other men or women [20]. The proportions of behaviors are likely to reflect many more acts in MSM, thus a greater chance for exposure to ESBL-E. Second, based on phylogenetic data, there is very little overlap between purported transmission networks of ESBL-E between MSM and other men or women [28]. Coupled with the higher prevalence of ESBL-E in MSM, the epidemiological context of ESBL-E transmission would be considered different to other men or women, hence influencing the association on behavior clusters and ESBL-E prevalence. Finally, it could be that other mechanisms of ESBL-E acquisition were more likely at play in other men or women, namely travel to regions with high ESBL-E prevalence [11,31].

To the best of our knowledge, this study is one of the first to study the relationship between sexual behaviors and ESBL-E carriage in sexual groups other than strictly MSM. The detailed questionnaire allowed us to pinpoint sexual behaviors that are associated with ESBL-E carriage and to add further evidence of how sexual contact could favor transmission of ESBL-E carriage. The large numbers of included individuals also permitted a wide range of sexual behaviors, from which we were able to differentiate different levels of risk of ESBL-E carriage. Taken these advantages into consideration, these results could help guide which empirical antibiotics should be given in groups with high risk of ESBL-E carriage.

Nevertheless, this study has certain limitations. First, the study pertains to only individuals who are at risk of acquiring STIs and caution should be heeded when interpreting results to the broader population. Second, we relied on self-reported sexual behaviors, which could be prone to memory bias. The questionnaire was also administered with an interviewer and as questions pertained to sensitive information about sexual behaviors, responses could have been biased by non-reporting (e.g. from fear of stigmatization) or by over reporting (e.g., from social desirability bias). Third, we did not include behaviors involving women (i.e., active cunnilingus, insertive vaginal sex) in the LCA for MSM who also have sex with women. Different classes of behaviors could have been observed because of adding these behaviors. However, transmission of ESBL-E from these behaviors would be unlikely, given the analysis on other men, and thus their inclusion would have only induced more statistical variation. Finally, we did not ask questions related to specific activities during travel or the reasons for being hospitalized, which could have been potential confounders [10,31].

In conclusion, our analysis was able to identify seven clusters of sexual behaviors among MSM at risk of acquiring STIs, three of which were associated with a higher prevalence of ESBL-E carriage compared to a cluster with few sexual behaviors. Clusters of sexual behaviors were also observed in other men and women, but no clear association with higher ESBL-E prevalence could be established. These data support the hypothesis that ESBL-E can be sexually transmitted, but transmission is likely dependent on sexual behaviors associated with fecal-oral routes and the epidemiological context of the key population. These data could be useful in determining empirical antibiotic therapy and suggesting measures to prevent further transmission of ESBL-E, especially to populations susceptible to these infections.

CONFLICT OF INTEREST

A.B. received speaker's fees from Gilead Sciences, Inc. T. C. received travel grants from Merck Sharp & Dohme (MSD), Eumedica, Gilead Sciences, ViiV Healthcare, Pfizer PFE France, INC Research, and Janssen-Cilag. P. L. W. received personal fees for participating on advisory boards from MSD. K. L. received travel grants and personal fees for participating on advisory boards and educational activities from MSD, ViiV healthcare, and Gilead, outside of this scope. L. S. received travel grant from Pfizer, outside the scope of this work. All other authors report no potential conflicts.

FUNDING SOURCE

This work was supported by Agence Nationale de Recherches sur le Sida et les Hépatites Virales (ANRS) (grant ECTZ62340).

ETHICAL APPROVAL STATEMENT

This study was approved by the "*Comité de protection des personnes Ouest II*" ethics committee (2017-73) in accordance with the Helsinki Declaration. All participants were required to provide consent for participation in the study and for the use of their data in analysis. This study was conform with the European Union's General Data Protection Regulation.

ACKNOWLEDGEMENTS

We thank all participants in the study. We particularly thank Elise Richet, Ysatis Espie, Valérie Lalande, and Salah Galah for their assistance with study design, data collection, and data management. The *Institut de Médecine et d'Epidémiologie Appliquée* (Paris, France) was responsible for coordinating the study.

REFERENCES

[1] Pitout JDD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis **2008**; 8:159–66.

[2] Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. J Antimicrob Chemother **2007**; 60:913–20.

[3] Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y. Clinical and economic impact of bacteremia with extended- spectrum-beta-lactamase-producing Enterobacteriaceae. Antimicrob Agents Chemother **2006**; 50:1257–62.

[4] Ruppé E, Lixandru B, Cojocaru R, et al. Relative Fecal Abundance of Extended-Spectrum-β-Lactamase-Producing Escherichia coli Strains and Their Occurrence in Urinary Tract Infections in Women. Antimicrob Agents Chemother **2013**; 57:4512–4517.

[5] Neulier C, Birgand G, Ruppé É, et al. Enterobacteriaceae bacteremia: Risk factors for ESBLPE. Med Mal Infect **2014**; 44:32–38.

[6] Bezabih YM, Bezabih A, Dion M, et al. Comparison of the global prevalence and trend of human intestinal carriage of ESBL-producing Escherichia coli between healthcare and community settings: a systematic review and meta-analysis. JAC Antimicrob Resist **2022**; 4:dlac048.

[7] Nicolas-chanoine MH, Gruson C, Bialek-Davenet S, et al. 10-fold increase (2006-11) in the rate of healthy subjects with extended-spectrum β-lactamase-producing Escherichia coli faecal carriage in a parisian check-up centre. Journal of Antimicrobial Chemotherapy **2013**; 68:562–568.

[8] Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal Colonization With Extended-spectrum Beta-lactamase-Producing Enterobacteriaceae and Risk Factors Among Healthy Individuals: A Systematic Review and Metaanalysis. Clin Infect Dis **2016**; 63:310–8.

[9] Osthoff M, McGuinness SL, Wagen AZ, Eisen DP. Urinary tract infections due to extended-spectrum beta-lactamase-producing Gram-negative bacteria: identification of risk factors and outcome predictors in an Australian tertiary referral hospital. Int J Infect Dis **2015**; 34:79–83.

[10] Hagel S, Makarewicz O, Hartung A, et al. ESBL colonization and acquisition in a hospital population: The molecular epidemiology and transmission of resistance genes. PLoS One **2019**; 14:e0208505.

[11] Arcilla MS, van Hattem JM, Haverkate MR, et al. Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet Infect Dis **2017**; 17:78–85.

[12] Cochard H, Aubier B, Quentin R, van der Mee-Marquet N, Réseau des Hygiénistes du Centre. Extended-spectrum β-lactamase-producing Enterobacteriaceae in French nursing

homes: an association between high carriage rate among residents, environmental contamination, poor conformity with good hygiene practice, and putative resident-to-resident transmission. Infect Control Hosp Epidemiol **2014**; 35:384–9.

[13] HCSP. Recommandations relatives aux mesures à mettre en oeuvre pour prévenir l'émergence des entérobactéries BLSE et lutter contre leur dissémination. 2010.

[14] Aragón TJ, Vugia DJ, Shallow S, et al. Case-control study of shigellosis in San Francisco: the role of sexual transmission and HIV infection. Clin Infect Dis **2007**; 44:327–34.

[15] Mook P, McCormick J, Bains M, et al. ESBL-Producing and Macrolide-Resistant Shigella sonnei Infections among Men Who Have Sex with Men, England, 2015. Emerg Infect Dis **2016**; 22:1948–1952.

[16] Jacqueline C, Carrascoso GR, Gutiérrez-Fernández J, et al. Genetic Characterization of Extensively Drug-Resistant Shigella sonnei Infections, Spain, 2021-2022. Emerg Infect Dis **2023**; 29:2370–2373.

[17] Ndumbi P, Freidl GS, Williams CJ, et al. Hepatitis A outbreak disproportionately affecting men who have sex with men (MSM) in the European Union and European Economic Area, June 2016 to May 2017. Euro Surveill **2018**; 23.

[18] Gaudreau C, Rodrigues-Coutlée S, Pilon PA, Coutlée F, Bekal S. Long-Lasting Outbreak of Erythromycin- and Ciprofloxacin-Resistant Campylobacter jejuni Subspecies jejuni from 2003 to 2013 in Men Who Have Sex with Men, Quebec, Canada. Clinical Infectious Diseases **2015**; 61:1549–1552.

[19] Gaudreau C, Pilon PA, Sylvestre J-L, Boucher F, Bekal S. Multidrug-Resistant Campylobacter coli in Men Who Have Sex with Men, Quebec, Canada, 2015. Emerg Infect Dis **2016**; 22:1661–3.

[20] Surgers L, Chiarabini T, Royer G, et al. Evidence of Sexual Transmission of Extended-Spectrum β-Lactamase-Producing Enterobacterales: A Cross-sectional and Prospective Study. Clin Infect Dis **2022**; 75:1556–1564.

[21] van Bilsen WPH, van Dulm E, Matser A, et al. High carriage of ESBL-producing Enterobacteriaceae associated with sexual activity among men who have sex with men. Int J Antimicrob Agents **2021**; 57.

[22] Boyd A, El Dani M, Ajrouche R, et al. Gut microbiome diversity and composition in individuals with and without extended-spectrum β-lactamase-producing Enterobacterales carriage: a matched case-control study in infectious diseases department. Clin Microbiol Infect **2024**;

[23] White A, Murphy TB. **BayesLCA** : An *R* Package for Bayesian Latent Class Analysis. J Stat Softw **2014**; 61.

[24] Reuland EA, Al Naiemi N, Kaiser AM, et al. Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in Amsterdam. J Antimicrob Chemother **2016**; 71:1076–82.

[25] Simms I, Gilbart VL, Byrne L, et al. Identification of verocytotoxin-producing Escherichia coli O117:H7 in men who have sex with men, England, November 2013 to August 2014. Euro Surveill **2014**; 19.

[26] Tansarli GS, Long DR, Waalkes A, et al. Genomic reconstruction and directed interventions in a multidrug-resistant Shigellosis outbreak in Seattle, WA, USA: a genomic surveillance study. Lancet Infect Dis **2023**; 23:740–750.

[27] Vecilla DF, Urrutikoetxea Gutiérrez MJ, Nieto Toboso MC, et al. First report of Shigella sonnei carrying a blaCTX-M-15 sexually transmitted among men who have sex with men. Infection **2024**;

[28] de Korne-Elenbaas J, van der Putten BCL, Boek NDM, et al. Putative transmission of extended-spectrum β-lactamase-producing Escherichia coli among men who have sex with men in Amsterdam, the Netherlands. Int J Antimicrob Agents **2023**; 62:106810.

[29] Campos-Madueno EI, Moradi M, Eddoubaji Y, et al. Intestinal colonization with multidrug-resistant Enterobacterales: screening, epidemiology, clinical impact, and strategies to

decolonize carriers. Eur J Clin Microbiol Infect Dis **2023**; 42:229–254.

[30] Slurink IAL, van Benthem BHB, van Rooijen MS, Achterbergh RCA, van Aar F. Latent classes of sexual risk and corresponding STI and HIV positivity among MSM attending centres for sexual health in the Netherlands. Sex Transm Infect **2020**; 96:33–39.

[31] Ruppé E, Armand-Lefèvre L, Estellat C, et al. High Rate of Acquisition but Short Duration of Carriage of Multidrug-Resistant Enterobacteriaceae After Travel to the Tropics. Clin Infect Dis **2015**; 61:593–600.

Table 1. Characteristics of the study population stratified on sexual group

	Sexual group			p[a]
	MSM (*n*=1211)	Other men (*n*=439)	Women (*n*=479)	
Age, median (IQR), y	38 (30-48)	27 (23-33)	25 (22-29)	<0.001
Geographic origin[b]				<0.001
Europe	980 (80.9%)	348 (79.3%)	392 (82.0%)	
North America, DROM-COM or Australia	113 (9.3%)	23 (5.2%)	33 (6.9%)	
Africa or Middle East	92 (7.6%)	64 (14.6%)	46 (9.6%)	
Asia	26 (2.1%)	4 (0.9%)	7 (1.5%)	
Born in France	932 (77.0 %)	352 (80.2 %)	393 (82.0%)	0.051
Communal living[c]	16 (1.3%)	11 (2.5%)	15 (3.1%)	0.036
Antibiotic use in past 6 months	402 (33.2%)	47 (10.7%)	84 (17.5%)	<0.001
Type of antibiotic used				<0.001
None	809 (66.8%)	392 (89.3%)	395 (82.5%)	
β-Lactams	131 (10.8%)	26 (5.9%)	48 (10.0%)	
Doxycline	64 (5.3%)	10 (2.3%)	9 (1.9%)	
Other	207 (17.1%)	11 (2.5%)	27 (5.6%)	
Antibiotic use in past 3 months	273 (22.5%)	30 (6.8%)	62 (12.9%)	<0.001
Travel outside of France	887 (73.2%)	313 (71.3%)	357 (74.5%)	0.54
Hospitalization in the past 12 months	69 (5.7%)	11 (2.5%)	19 (4.0%)	0.018
Number of sexual partners in the past 6 months				<0.001
0-7	610 (50.4%)	402 (91.6 %)	449 (93.7%)	
8-30	436 (36.0%)	34 (7.7 %)	30 (6.3%)	
>30	165 (13.6%)	3 (0.7 %)	0 (0.0%)	
HIV positive	489 (40.4%)	0 (0.0%)	0 (0.0%)	*ntp*
PrEP use in the past 6 months[d]	246 (34.1%)	1 (0.2%)	1 (0.2%)	<0.001
Any condomless sex in the past 6 months	1023 (84.5%)	371 (84.5%)	396 (82.7%)	0.63
Drug use[e] in the past 6 months	535 (44.2%)	161 (36.7%)	170 (35.5%)	<0.001
Any STI in the past 6 months	315 (26.0%)	26 (5.9%)	28 (5.8%)	<0.001
Sex work in the past 6 months	29 (2.4%)	7 (1.6%)	5 (1.0%)	0.16

Data were obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019. Data represent no. (%) of participants unless otherwise identified as median (IQR).

[a]Statistical comparisons were performed using Pearson χ^2 or Fisher exact test for categorical and Kruskal-Wallis test for continuous variables.

[b]Missing value for 1 participant in the women group.

[c]Communal living defined as living in a hotel, camp, or shelter or experiencing homelessness.

[d]Among participants without HIV.

[e]Drug use excludes alcohol and tobacco use.

Abbreviations: DROM-COM, *départements et régions d'outre-mer et collectivités d'outre-mer* (Overseas France ou French overseas territories); ESBL-E, extended-spectrum β-lactamase–

producing Enterobacterales; HIV, human immunodeficiency virus; IQR, interquartile range; *ntp*, no test performed; PrEP, pre-exposure prophylaxis for HIV; STI, sexually transmitted infection.

Table 2. Description of sexual behaviors stratified on sexual group

Sexual behaviors[a]	Sexual group			p[b]
	MSM (*n*=1211)	Other men (*n*=439)	Women (*n*=479)	
Receptive anal intercouse	969 (80.0%)	-	160 (33.4%)	<0.001
Insertive anal intercourse	957 (79.0%)	154 (35.1%)	-	<0.001
Passive rimming	727 (60.0%)	42 (9.6%)	86 (18.0%)	<0.001
Active rimming	697 (57.6%)	94 (21.0%)	47 (9.8%)	<0.001
Passive cunnilingus	-	-	345 (72.0%)	*ntp*
Active cunnilingus	-	322 (73.3%)	-	*ntp*
Receptive fellatio	1071 (88.4%)	362 (82.5%)	-	<0.001
Insertive fellatio	1077 (88.9%)	-	408 (85.2%)	<0.001
Receptive fisting	104 (8.6%)	-	8 (1.7%)	<0.001
Insertive fisting	171 (14.1%)	5 (1.1%)	-	<0.001
Receptive fingering	611 (50.5%)	42 (9.6%)	388 (81.0%)	<0.001
Insertive fingering	704 (58.1%)	365 (83.1%)	83 (17.3%)	<0.001

Data were obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019. Data represent no. (%) of participants.

[a]Insertive and receptive vaginal intercourse was not included, as these behaviors were not considered in the Bayesian latent class analysis.

[b]Statistical comparisons were performed using Pearson χ^2 or Fisher exact test for categorical and Kruskal-Wallis test for continuous variables.

Abbreviations: MSM, men who have sex with men; *ntp*, no test performed.

Table 3. Association between individual sexual behaviors and ESBL-E carriage, stratified by sexual group

Sexual behavior[a]	ESBL-E carriage, *n* (%)		Non-adjusted		Adjusted[b]	
	No	Yes	OR (95%CI)	*P*	aOR (95%CI)	*P*
MSM (*N*=1211)	(*n*=1062)	(*n*=149)				
Receptive anal intercouse	840 (79.1%)	129 (86.6%)	1.70 (1.06-2.87)	0.034	1.60 (0.99-2.71)	0.063
Insertive anal intercourse	842 (79.3%)	115 (77.2%)	0.88 (0.59-1.35)	0.56	0.79 (0.52-1.21)	0.26
Passive rimming	630 (59.3%)	97 (65.1%)	1.28 (0.90-1.84)	0.18	1.04 (0.72-1.52)	0.83
Active rimming	608 (57.3%)	89 (59.7%)	1.11 (0.78-1.58)	0.57	0.92 (0.64-1.33)	0.65
Receptive fellatio	934 (87.9%)	137 (91.9%)	1.56 (0.88-3.05)	0.16	1.34 (0.74-2.64)	0.36
Insertive fellatio	944 (88.9%)	133 (89.3%)	1.04 (0.61-1.87)	0.89	0.89 (0.52-1.62)	0.69
Receptive fisting	81 (7.6%)	23 (15.4%)	2.21 (1.32-3.59)	0.002	1.99 (1.17-3.26)	0.008
Insertive fisting	142 (13.4%)	29 (19.5%)	1.57 (0.99-2.41)	0.047	1.41 (0.88-2.19)	0.14
Receptive fingering	83 (55.7%)	528 (49.7%)	1.27 (0.90-1.80)	0.17	1.14 (0.80-1.62)	0.47
Insertive fingering	611 (57.5%)	93 (62.4%)	1.23 (0.86-1.75)	0.26	1.06 (0.74-1.53)	0.76
Other men (*N*=439)	(*n*=395)	(*n*=44)				
Insertive anal intercourse	142 (35.9%)	12 (27.3%)	0.67 (0.32-1.31)	0.26	0.69 (0.33-1.36)	0.30
Passive rimming	37 (9.4%)	5 (11.4%)	1.24 (0.41-3.09)	0.67	1.29 (0.42-3.23)	0.61
Active rimming	82 (20.8%)	10 (22.7%)	1.12 (0.51-2.29)	0.76	1.16 (0.52-2.39)	0.70
Active cunnilingus	291 (73.7%)	31 (70.5%)	0.85 (0.44-1.74)	0.65	0.86 (0.44-1.76)	0.66
Receptive fellatio	331 (83.8%)	31 (70.5%)	0.46 (0.23-0.96)	0.030	0.46 (0.23-0.96)	0.030
Insertive fisting	5 (1.3%)	0	[c]		[c]	
Receptive fingering	41 (10.4%)	1 (2.3%)	0.20 (0.01-0.96)	0.12	0.20 (0.01-0.94)	0.11
Insertive fingering	333 (84.3%)	32 (72.7%)	0.50 (0.25-1.05)	0.056	0.50 (0.25-1.05)	0.056
Women (*N*=479)	(*n*=446)	(*n*=33)				
Receptive anal sex	148 (33.2%)	12 (36.4%)	1.15 (0.54-2.37)	0.71	1.25 (0.57-2.61)	0.56
Active rimming	83 (18.6%)	3 (9.1%)	0.44 (0.10-1.27)	0.18	0.48 (0.11-1.43)	0.25
Passive rimming	47 (10.5%)	0	[c]		[c]	
Receptive cunnilingus	322 (72.2%)	23 (69.7%)	0.89 (0.42-2.00)	0.76	0.93 (0.43-2.12)	0.85
Insertive fellatio	379 (85.0%)	29 (87.9%)	1.28 (0.48-4.42)	0.65	1.21 (0.45-4.23)	0.73
Receptive fisting	7 (1.6%)	1 (3.0%)	1.96 (0.10-11.50)	0.54	2.84 (0.14-18.90)	0.36
Receptive fingering	363 (81.4%)	25 (75.8%)	0.71 (0.32-1.74)	0.43	0.73 (0.33-1.80)	0.46
Insertive fingering	77 (17.3%)	6 (18.2%)	1.06 (0.39-2.51)	0.89	1.23 (0.44-2.99)	0.66

Data were obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019.

[a]Insertive and receptive vaginal intercourse was not included, as these behaviors were not considered in the Bayesian latent class analysis.

[b]Adjusted for antibiotic use in the past 6 months, highest ESBL-E prevalence of country travelled (i.e., none, low, medium, high), and number of sexual partners (i.e., 0-7, 8-30, ≥30 for MSM and 0-7 and ≥8 for other men and women).

[c]Parameter estimates were not obtained due to 0 prevalence in certain categories.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; ESBL-E, Extended-Spectrum β-Lactamase–Producing Enterobacterales; MSM, men who have sex with men; OR, odds ratio.

Table 4. Association between latent classes and ESBL-E carriage, stratified by sexual group

Latent class[a]	n	Mean proportion of behaviors	ESBL-E prevalence	Non-adjusted		Adjusted[b]	
				OR (95%CI)	*P*	aOR (95%CI)	*P*
MSM (*N*=1211)							
Class 1	259	29.9%	7.3%	Ref.		Ref.	
Class 2	102	42.3%	12.7%	1.85 (0.86-3.86)	0.11	1.74 (0.81-3.66)	0.15
Class 3	124	44.8%	15.3%	2.29 (1.16-4.52)	0.017	2.19 (1.11-4.34)	0.023
Class 4	100	60.0%	16.0%	2.41 (1.17-4.89)	0.015	2.31 (1.12-4.72)	0.021
Class 5	198	62.2%	10.6%	1.50 (0.78-2.89)	0.22	1.41 (0.73-2.73)	0.31
Class 6	358	79.7%	12.3%	1.77 (1.02-3.18)	0.047	1.65 (0.95-2.97)	0.084
Class 7	70	91.7%	24.3%	4.05 (1.96-8.34)	<0.001	3.78 (1.81-7.83)	<0.001
Other men (*N*=439)							
Class 1	72	11.1%	16.7%	Ref		Ref	
Class 2	297	45.0%	8.1%	0.44 (0.21-0.95)	0.031	0.44 (0.21-0.96)	0.031
Class 3	70	79.0%	11.4%	0.65 (0.24-1.67)	0.37	0.64 (0.24-1.67)	0.37
Women (*N*=479)							
Class 1	64	7.6%	9.4%	Ref		Ref	
Class 2	343	44.7%	7.0%	0.73 (0.30-2.03)	0.51	0.72 (0.29-2.04)	0.50
Class 3	72	81.2%	4.2%	0.42 (0.09-1.67)	0.23	0.45 (0.09-1.82)	0.28

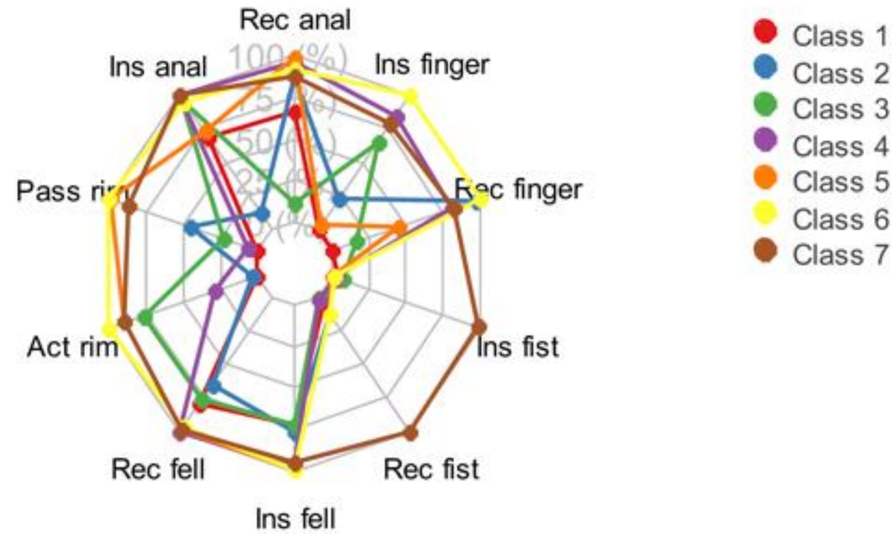
Data were obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019.

[a]Composition of latent classes for MSM, other men and women are described in Figures 1A, 1B, and 1C, respectively.

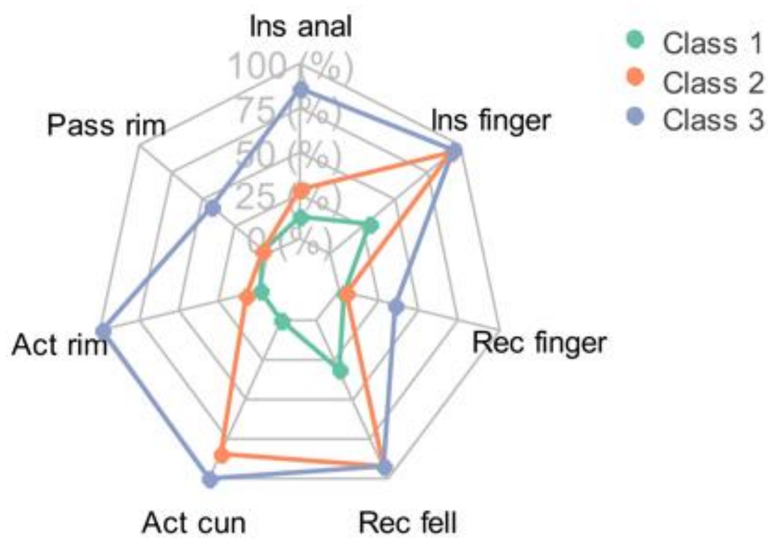
[b]Adjusted for antibiotic use in the past 6 months and highest ESBL-E prevalence of country travelled (i.e., none, low, medium, high).

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; ESBL-E, Extended-Spectrum β-Lactamase–Producing Enterobacterales; OR, odds ratio; ref, reference group.

A


Rec anal
Ins finger
Rec finger
Ins fist
Rec fist
Ins fell
Rec fell
Act rim
Pass rim
Ins anal
100 (%)
75 (%)
50 (%)
25 (%)
0 (%)
Class 1
Class 2
Class 3
Class 4
Class 5
Class 6
Class 7

B


Ins anal
Ins finger
Rec finger
Rec fell
Act cun
Act rim
Pass rim
100 (%)
75 (%)
50 (%)
25 (%)
0 (%)
Class 1
Class 2
Class 3

C

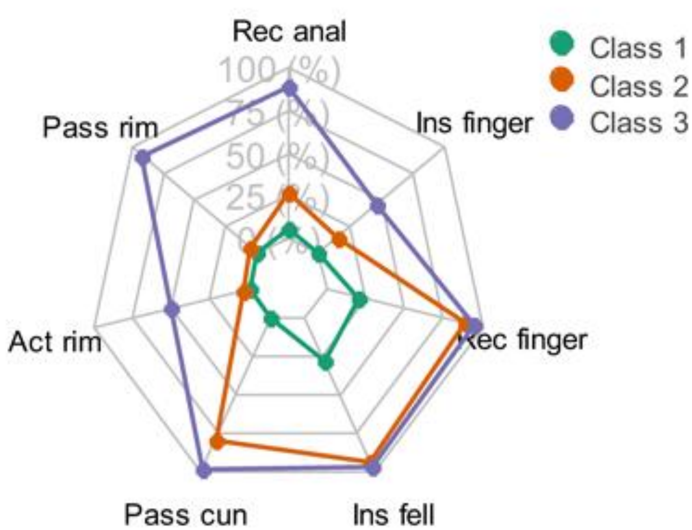

Rec anal
Ins finger
Rec finger
Ins fell
Pass cun
Act rim
Pass rim
100 (%)
75 (%)
50 (%)
25 (%)
0 (%)
Class 1
Class 2
Class 3

FIGURE LEGENDS

Figure 1. Description of sexual behavior clusters

Plots depict the proportion (in %) of individuals engaging in a given sexual behavior in the last 6 months, according to latent class. Analysis is stratified by men who have sex with men (**A**), other men (**B**), and women (**C**). Data were obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019.

Abbreviations: Act, active; cun, cunnilingus; fell, fellatio; fist, fisting; finger, fingering; Ins, insertive; Pass, passive; Rec, receptive; rim, rimming

Supplement to: Boyd A, Mathieu P, Françoise U, et al. Sexual behaviors and risk of extended-spectrum β-lactamase-producing Enterobacterales carriage: a cross-sectional analysis.

Table of contents

SUPPLEMENTARY METHODS

Determining presence of ESBL-E in rectal swab samples

To ensure detection of small bacterial loads, we grew three separate precultures in Brain Heart Infusion (BHI) broth with 10µg/ml cefotaxime (to select for ESBL-E) after inoculation from the same e-rectal swab. Precultures were grown for 18h at 37°C. We then plated each culture on bioMerieux ESBL medium for selective isolation of ESBL-E. After incubating plates for another 18h at 37°C, identification of bacterial colonies was performed using mass spectrometry (MALDI BiotyperR, Bruker, Billerica, MA, USA). We chose colonies of ESBL-E strains picked from selective media and stored them at -80°C for further analysis. To ensure that Enterobacterales exhibiting antibiotic resistance via indirect pathways (e.g., overproduction of AmpC) were not selected, all enterobacterial or enterococcal species isolates were subjected to a standard antibiogram using Mueller-Hinton agar (Biorad), according to recommendations from the European Committee on Antimicrobial Susceptibility Testing (https://www.eucast.org/).

SUPPLEMENTARY TABLES

Supplementary Table S1. Fit statistics for Bayesian latent class models according to sexual group

Number of classes (*k*)	MSM		Other men		Women	
	AIC	BIC	AIC	BIC	AIC	BIC
2	-11004	-11111	-2685	-2746	-3027	-3090
3	-10692	-10855	-2579	-2673	-2920	-3016
4	-10530	-10750	-2573	-2700	-2900	-3029
5	-10365	-10640	-2570	-2729	-2891	-3054
6	-10221	-10552	-2569	-2761	-2888	-3085
7	-10145	-10532	-2571	-2796	-2887	-3137
8	-10089	-10533	-2577	-2835	-2892	-3154

Latent class models were fit, as described in the Statistical analysis section, on data obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019. Models were fit to increasing numbers of classes (*k*). The Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) were calculated and the model with the BIC closest to 0 was selected in further analysis.

Supplementary Table S2. Characteristics of the study population stratified on membership of sexual behavior cluster (in men who have sex with men)

	Sexual clusters							*p*[a]
	Cluster 1 (*n*=259)	Cluster 2 (*n*=102)	Cluster 3 (*n*=124)	Cluster 4 (*n*=100)	Cluster 5 (*n*=198)	Cluster 6 (*n*=358)	Cluster 7 (*n*=70)	
Age, median (IQR), y	46 (34-53)	39 (28-47)	34 (28-46)	38 (30-45)	37 (30-45)	35 (28-45)	42 (35-49)	<0.001
Geographic origin								<0.001
Europe	185 (71.4%)	75 (73.5%)	100 (80.6%)	82 (82.0%)	164 (82.8%)	310 (86.6%)	64 (91.4%)	
North America, DOM, TOM or Australia	32 (12.4%)	8 (7.8%)	13 (10.5%)	10 (10.0%)	20 (10.1%)	28 (7.8%)	2 (2.9%)	
Africa or Middle East	32 (12.4%)	16 (15.7%)	9 (7.3%)	6 (6.0%)	11 (5.6%)	15 (4.2%)	3 (4.3%)	
Asia	10 (3.9%)	3 (2.9%)	2 (1.6%)	2 (2.0%)	3 (1.5%)	5 (1.4%)	1 (1.4%)	
Born in France	179 (69.1 %)	74 (72.5 %)	96 (77.4%)	76 (76.0%)	152 (76.8%)	296 (82.7%)	59 (84.3%)	0.004
Communal living[b]	9 (3.5%)	2 (2.0%)	2 (1.6%)	1 (1.0%)	1 (0.5%)	1 (0.3%)	0 (0)	0.023
Antibiotic use in past 6 months	58 (22.4%)	33 (32.4%)	42 (33.9%)	31 (31.0%)	71 (35.9%)	133 (37.2%)	34 (48.6%)	<0.001
Type of antibiotic used								0.0020
None	201 (77.6%)	69 (67.6%)	82 (66.1%)	69 (60.0%)	127 (64.1%)	225 (62.8%)	36 (51.4%)	
β-Lactams	29 (11.2%)	11 (10.8%)	13 (10.5%)	9 (9.0%)	22 (11.1%)	38 (10.6%)	9 (12.9%)	
Doxycline	10 (3.9%)	7 (3.9%)	3 (6.9%)	2 (2.0%)	14 (7.1%)	22 (6.1%)	6 (8.6%)	
Other	19 (7.3%)	15 (7.3%)	26 (14.7%)	20 (20.0%)	35 (17.7%)	73 (20.4%)	19 (27.1%)	
Antibiotic use in past 3 months	40 (15.4%)	21 (20.6%)	21 (16.9%)	24 (24.0%)	50 (25.3%)	91 (25.4%)	26 (37.1%)	0.0017
Travel outside of France	171 (66.0%)	77 (75.5%)	90 (72.6%)	73 (73.0%)	147 (74.2%)	276 (77.1%)	53 (75.7%)	0.17
Hospitalization in the past 12 months	14 (5.4%)	1 (1.0%)	8 (6.5%)	8 (8.0%)	12 (6.1%)	22 (6.1%)	4 (5.7%)	0.47
Number of sexual partners in the past 6 months								<0.001
0-7	187 (72.2%)	60 (58.8%)	63 (50.8%)	52 (52.0%)	81 (40.9%)	151 (42.2%)	16 (22.9%)	
8-30	61 (23.6%)	31 (30.4%)	45 (36.3%)	32 (32.0%)	83 (41.9%)	145 (40.5%)	39 (55.7%)	
>30	11 (4.2%)	11 (10.8%)	16 (10.8%)	16 (16.0%)	34 (17.2%)	62 (17.3%)	15 (21.4%)	
HIV positive	167 (64.4%)	27 (26.4%)	52 (41.9%)	33 (33.0%)	64 (32.3%)	109 (30.4%)	37 (52.9%)	<0.001
PrEP use in the past 6 months[c]	32 (34.8%)	18 (24.0%)	24 (33.3%)	30 (44.8%)	46 (34.3%)	79 (31.7%)	17 (51.5%)	<0.001
Condomless sex in the past 6 months	199 (76.8%)	79 (77.5%)	109 (87.9%)	89 (89.0%)	171 (86.4%)	321 (89.7%)	55 (78.6%)	<0.001
Drug use[d] in the past 6 months	69 (26.6%)	40 (39.2%)	56 (45.2%)	40 (40.0%)	97 (49.0%)	189 (52.8%)	44 (62.9%)	<0.001
Any STI in the past 6 months	55 (21.2%)	28 (27.5%)	36 (29.0%)	26 (26.0%)	52 (26.3%)	93 (26.0%)	25 (35.7%)	0.30
Sex work in the past 6 months	3 (1.2%)	0 (0%)	6 (4.8%)	3 (3.0%)	7 (3.5%)	8 (2.2%)	2 (2.9%)	0.19

Data were obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019. Data represent no. (%) of participants unless otherwise identified as median (IQR). Composition of latent classes for MSM are described in Figure 1A.

[a]Statistical comparisons were performed using Pearson χ^2 or Fisher exact test for categorical and Kruskal-Wallis test for continuous variables.

[b]Communal living defined as living in a hotel, camp, or shelter or experiencing homelessness.

[c]Among participants without HIV.

[d]Drug use excludes alcohol and tobacco use.

Supplementary Table S3. Characteristics of the study population stratified on membership of sexual behavior cluster (in other men)

	Sexual clusters			p^a
	Cluster 1 (*n*=72)	Cluster 2 (*n*=297)	Cluster 3 (*n*=70)	
Age, median (IQR), y	28 (24-35)	26 (23-32)	29 (25-38)	0.0040
Geographic origin				0.0045
Europe	47 (65.3%)	245 (82.5%)	56 (80.0%)	
North America, DOM, TOM or Australia	3 (4.2%)	15 (5.1%)	5 (7.1%)	
Africa or Middle East	22 (30.6%)	34 (11.4%)	8 (11.4%)	
Asia	0 (0%)	3 (1.0%)	1 (1.4%)	
Born in France	47 (65.3%)	249 (83.8%)	56 (80.0%)	0.0019
Communal living[b]	7 (9.7%)	3 (1.0%)	1 (1.4%)	0.0001
Antibiotic use in past 6 months	9 (12.5%)	29 (9.8%)	9 (12.9%)	0.65
Type of antibiotic used				0.41
None	63 (87.5%)	268 (90.2%)	61 (87.1%)	
β-Lactams	3 (4.2%)	18 (6.1%)	5 (7.1%)	
Doxycline	2 (2.8%)	7 (2.4%)	4 (1.4%)	
Other	4 (5.6%)	4 (1.3%)	3 (4.3%)	
Antibiotic use in past 3 months	6 (8.3%)	17 (5.7%)	7 (10.0%)	0.38
Travel outside of France	48 (66.7%)	215 (72.4%)	50 (71.4%)	0.63
Hospitalization in the past 12 months	1 (1.4%)	8 (2.7%)	2 (2.9%)	0.80
Number of sexual partners in the past 6 months				<0.0001
0-7	68 (94.4%)	278 (93.6%)	56 (80.0%)	
8-30	4 (5.6%)	19 (6.4%)	11 (15.7%)	
>30	0 (0%)	0 (0%)	3 (4.3%)	
HIV positive	0 (0%)	0 (0%)	0 (0%)	*ntp*
PrEP use in the past 6 months[c]	1 (1.4%)	0 (0%)	0 (0%)	*ntp*
Condomless sex in the past 6 months	54 (75.0%)	257 (86.5%)	60 (85.7%)	0.050
Drug use[d] in the past 6 months	15 (20.8%)	108 (36.4%)	38 (54.3%)	0.0002
Any STI in the past 6 months	0 (0%)	21 (7.1%)	5 (7.1%)	0.066
Sex work in the past 6 months	0 (0%)	5 (1.7%)	2 (2.9%)	0.39

Data were obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019. Data represent no. (%) of participants unless otherwise identified as median (IQR). Composition of latent classes for other men are described in Figure 1B.

[a]Statistical comparisons were performed using Pearson χ^2 or Fisher exact test for categorical and Kruskal-Wallis test for continuous variables. *ntp*, no test performed.

[b]Communal living defined as living in a hotel, camp, or shelter or experiencing homelessness.

[c]Among participants without HIV.

[d]Drug use excludes alcohol and tobacco use.

Supplementary Table S4. Characteristics of the study population stratified on membership of sexual behavior cluster (in women)

	Sexual clusters			*p*[a]
	Cluster 1 (*n*=64)	Cluster 2 (*n*=343)	Cluster 3 (*n*=72)	
Age, median (IQR), y	26 (23-32)	24 (22-28)	25 (22-29)	0.016
Geographic origin				0.0012
Europe	42 (66.7%)	286 (83.4%)	64 (88.9%)	
North America, DOM, TOM or Australia	4 (6.3%)	23 (6.7%)	6 (8.3%)	
Africa or Middle East	15 (23.8%)	29 (8.5%)	2 (2.8%)	
Asia	2 (3.2%)	5 (1.5%)	0 (0%)	
Born in France	38 (59.4%)	295 (86.0%)	60 (83.3%)	<0.0001
Communal living[b]	7 (10.9%)	6 (1.7%)	2 (2.8%)	0.0005
Antibiotic use in past 6 months	14 (21.9%)	58 (16.9%)	12 (16.7%)	0.62
Type of antibiotic used				0.61
None	50 (78.1%)	285 (83.1%)	60 (83.3%)	
β-Lactams	8 (12.5%)	36 (10.5%)	4 (5.6%)	
Doxycline	2 (3.1%)	5 (1.5%)	2 (2.8%)	
Other	4 (6.3%)	17 (5.0%)	6 (8.3%)	
Antibiotic use in past 3 months	11 (17.2%)	41 (12.0%)	10 (13.9%)	0.50
Travel outside of France	43 (67.2%)	260 (75.8%)	54 (75.0%)	0.35
Hospitalization in the past 12 months	0 (0%)	15 (4.4%)	4 (5.6%)	0.20
Number of sexual partners in the past 6 months				*ntp*
0-7	62 (96.9%)	325 (94.8%)	62 (86.1%)	
8-30	2 (3.1%)	18 (5.2%)	10 (13.9%)	
>30	0 (0%)	0 (0%)	0 (0%)	
HIV positive	0 (0%)	0 (0%)	0 (0%)	*ntp*
PrEP use in the past 6 months[c]	0 (0%)	0 (0%)	1 (1.4%)	*ntp*
Condomless sex in the past 6 months	48 (75.0%)	285 (83.1%)	63 (87.5%)	0.15
Drug use[d] in the past 6 months	7 (10.9%)	127 (37.0%)	36 (50.0%)	<0.0001
Any STI in the past 6 months	2 (3.1%)	2 (0.6%)	1 (1.4%)	0.58
Sex work in the past 6 months	2 (3.1%)	2 (0.6%)	1 (1.4%)	0.18

Data were obtained from individuals in the BMR-IST cohort, Paris, France, 2018-2019. Data represent no. (%) of participants unless otherwise identified as median (IQR). Composition of latent classes for women are described in Figure 1C.

[a]Statistical comparisons were performed using Pearson χ^2 or Fisher exact test for categorical and Kruskal-Wallis test for continuous variables. *ntp*, no test performed.

[b]Communal living defined as living in a hotel, camp, or shelter or experiencing homelessness.

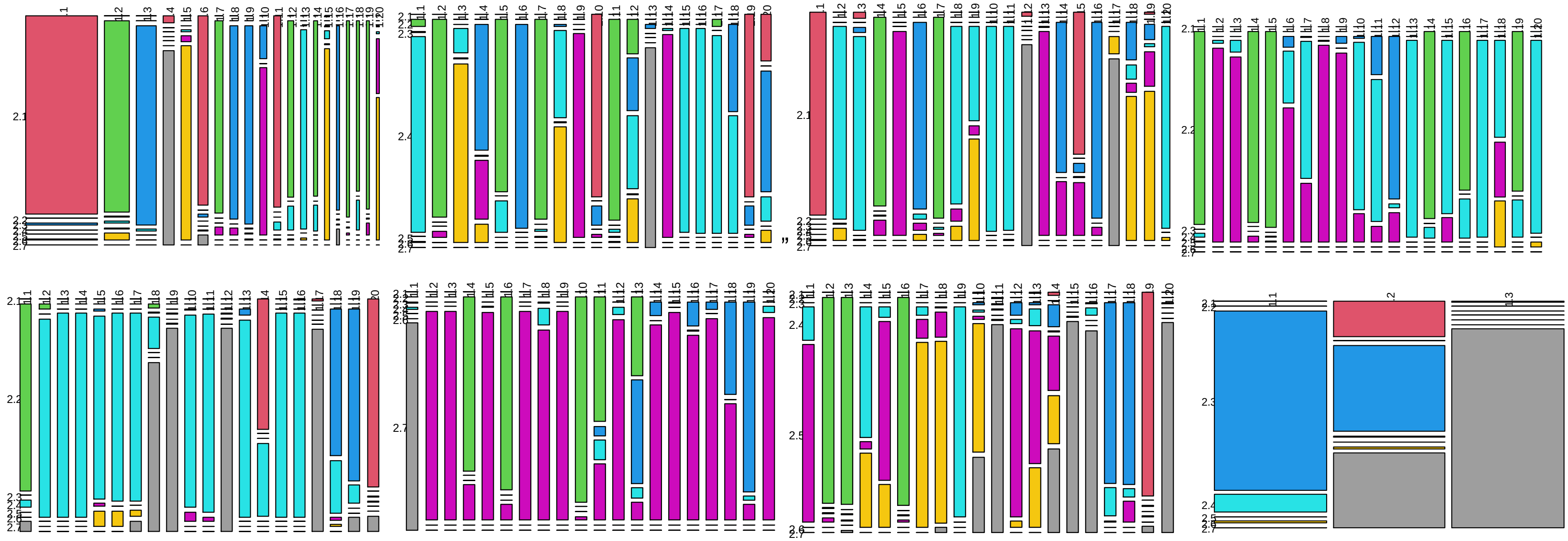
[c]Among participants without HIV.

[d]Drug use excludes alcohol and tobacco use.

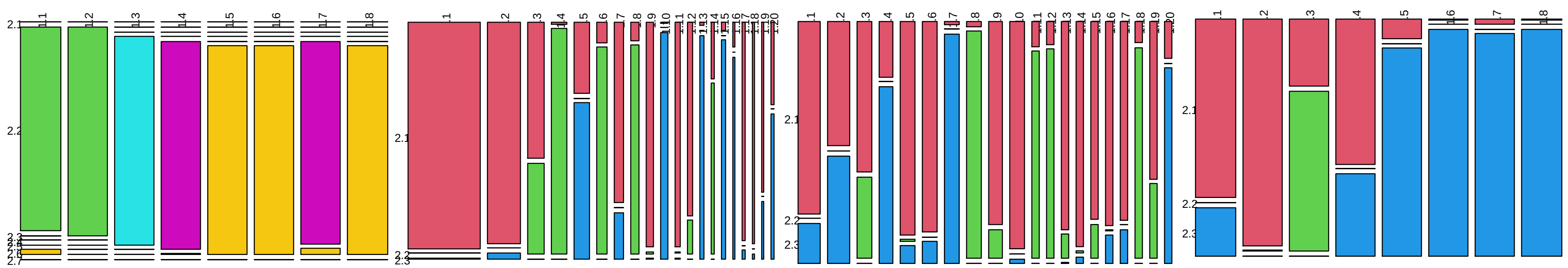
SUPPLEMENTARY FIGURES

Supplementary Figure S1. Certainty plots for Bayesian latent class models according to sexual group

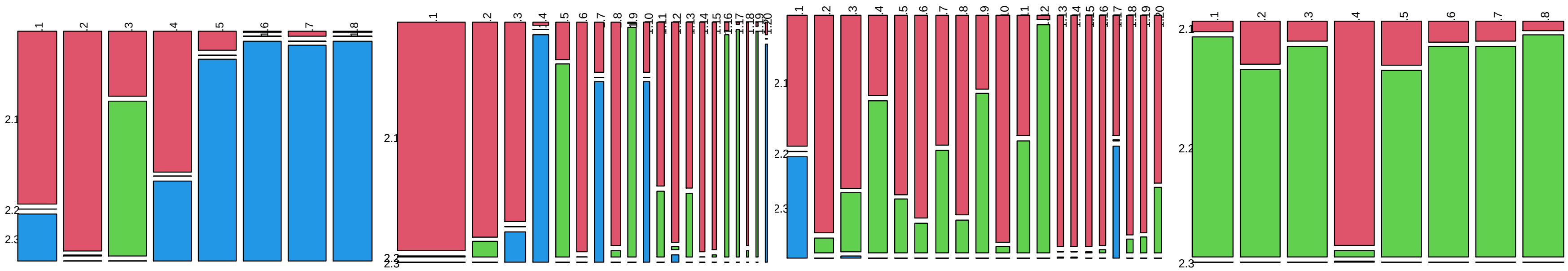
A



B



C



Plots are constructed in which combinations of sexual behaviors are categorized on the x-axis and the probability of belonging to each class is depicted on the y-axis. High degree of certainty is present when most categories have bars with one color. Given the high numbers of combinations, these diagnostic plots had to be presented in several panels. Plots are given for men who have sex with men (**A**), other men (**B**), and women (**C**).