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Risk factors of multidrug-resistant gonococcal infections: an analysis of data from the sentinel surveillance network- Québec, Canada, 2017–2019

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SUMMARY

The emergence of antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is a worrying global phenomenon, with major implications for public health. This study, a cross-sectional analysis of an open cohort, explored the risk factors associated with multidrug-resistant (MDR) gonococcal infections in the province of Québec, Canada, using data from the sentinel surveillance network from 2017 to 2019. The data comprised sociodemographic, epidemiological, and clinical information, collected through self-administered questionnaires, direct case interviews and chart reviews coupled with laboratory AMR data for five antibiotics: azithromycin, tetracycline, ciprofloxacin, cefixime, and ceftriaxone. Missing data were handled using multiple imputation with chain equations (MICE); generalized estimating equations (GEE) were used to assess correlates of MDR. The analysis included 714 participants with a total of 775 cases. We observed very frequent resistance to ciprofloxacin (74.3%), while resistance to tetracycline and azithromycin was 19.9% and 21.3%, respectively. MDR was found in 12.5% of cases. Multivariate analysis indicated that having five or more sexual partners in the past two months (aPR=1.61, 95%CI: 1.07-2.41) was associated with MDR. In conclusion, our results show an association between the number of sexual partners and MDR, supporting the hypothesis that resistance can develop more rapidly in dense sexual networks. It is crucial to address the issue of circulating resistant strains through ongoing surveillance, research, and targeted interventions to manage and contain them before they become widespread.

Keywords: Gonococcal infections, Multidrug resistance, Québec, Surveillance

INTRODUCTION

Antimicrobial resistance (AMR) among *Neisseria gonorrheae* represents a major threat to effective therapies, complicating both clinical management and public health control. In 2020, estimates showed that 82.4 million people were newly infected with gonorrhea; showing the seriousness of the situation [1]. This observed persistent high incidence could be due to several factors and among others, we can mention: urban residence, sexual orientation, sexual intercourse without condom use, alcohol and illicit substance abuse, socioeconomic factors, low screening rate, cultural and practice-related factors (beliefs surrounding antibiotic use, availability of over-the-counter antibiotics, doctor-patient relationships) and last but not least, AMR of circulating strains [2]. Given this background, the WHO has warned of the emergence of non-treatable cases of gonorrhea in the near future [3]. Canada has seen an increase in gonococcal cases in most provinces. There has been an increase of 65.4% between 2010 (33.5 cases

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per 100,000 population) and 2015 (55.4 cases per 100,000 population) [4]. In 2021, there was a further major increase in gonococcal infection rates to 84.2 cases per 100,000 population [5]. In the province of Québec in particular, this problem requires closer attention as the incidence rate has increased from 11.7 per 100,000 population to 84.8 per 100,000 population between 2002 and 2022 [6]. A laboratory-based surveillance program has been in place in the province of Québec to monitor *N. gonorrhoeae* AMR. Isolates received as part of that surveillance represent approximately 20% of cases reported, and strains resistant to various antibiotics have been documented [7–10]. In addition, epidemiological and clinical data were available for a subset of isolates as part of a sentinel surveillance network. A common limitation in surveillance systems is the presence of incomplete data on key clinical, behavioural, and sociodemographic variables. The "missingness" in data can lead to biased estimates and possibly affect the study conclusions; yet deep methodological considerations around data completeness are rarely emphasized in applied surveillance studies.

Leveraging data from the sentinel network, an open cohort for gonococcal infection surveillance in Québec, we pursued two objectives in the present study: (1) to determine correlates of MDR *N. gonorrhoeae* infections, and (2) to compare analyses performed with and without multiple imputation of missing data in order to assess the robustness of findings.

METHODOLOGY

Study setting, design and participants

This study used data from the sentinel surveillance network, which was an open cohort for gonococcal infections, implemented in three regions of the province of Québec (Montréal, Montérégie and Nunavik), from September 2015 to March 2020. As previously described, individuals who tested positive for *N. gonorrhoeae* at one of the 26 sites of the sentinel surveillance network and were at least 14 years old at the time of the consultations were enrolled in the study after consent. The collection of sociodemographic, epidemiological, and clinical information was made possible by a combination of auto-administered questionnaires, direct case interviews, as well as chart reviews. More information on the design of the sentinel network and the laboratory procedures at the Laboratoire de santé publique du Québec (LSPQ), where all samples were sent, can be found in previous publications [7,10,11].

Data extraction and study variables

The laboratory findings obtained from the LSPQ were integrated with sociodemographic, epidemiological, and clinical information gathered through the sentinel surveillance network. Subsequently, the database underwent a denominalization process to safeguard the anonymity of patient data. For the purpose of this study, we extracted data collected from 2017 to 2019. In this study, we covered the resistance profile of five antibiotics: azithromycin, tetracycline and ciprofloxacin; and increased minimum inhibitory concentration (MIC) of 3rd generation cephalosporins (3GC), namely cefixime and ceftriaxone. Due to the very low prevalence of isolates showing decreased susceptibility to 3GC, the cut-off point was based on literature reviews, with the aim of examining the emergence of an increased MIC to the 3GC. The cut-offs used to define the resistance or increased MIC level to different antibiotics can be found in Table 1.

The main outcome was the MDR status of *N. gonorrhoeae isolates*. MDR was defined as 1) resistance to ciprofloxacin AND 2) resistance to tetracycline AND 3) either resistance to azithromycin OR increased MIC to 3GC (cefixime or ceftriaxone) [12].

Independent variables included basic sociodemographic characteristics (age, residency area, gender, etc.) as well as epidemiological information (number of sex partners in last 2 months, sex outside of the province, previous STBBIs, etc.)

Statistical analyses

Descriptive statistics of baseline characteristics as well as specific resistance levels were presented in frequency tables or figures with counts and percentages.

Using generalized estitmating equations (GEEs), regressions analyses (univariate and multivariable) were carried for assessing correlates of MDR. Results were presented in terms of prevalence ratios (PRs) and their 95% confidence intervals (CI) [13,14]. The final multivariate analysis included all collected variables identified *a priori* in the literature review. A p-value<0.05 was considered statistically significant. GEEs were used to account for correlation between multiple visits by same individuals and the resulting dependence on variance estimates. Multivariate adjusted prevalence ratio (aPR) were computed with log link, poisson distribution and robust "sandwich" variance estimator, which corrects for potential problems caused by overdispersion.

Another aspect of this study was that we compared two procedures for handling missing data. The first one, used in a previous study [7], involved adding a "missing indicator" for variables with more than 20% missing data, a simple but less ideal approach. The second procedure, which we employed here, was

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Table 1. Cut-offs of resistance or increased MIC to tested antibiotics

ANTIBIOTICS	Resistance	Increased MIC	REFERENCE
azithromycin	MIC ≥2 mg/L	-	[7,31,32]
cefixime	-	MIC ≥ 0.06 mg/L	[8,33]
ceftriaxone	-	MIC ≥ 0.03 mg/L	[8,34]
tetracycline	MIC ≥ 2 mg/L	-	[8,35]
ciprofloxacin	MIC ≥ 1 mg/L	-	[8,34]

MIC: minimal inhibitory concentration

multiple imputation using chained equations (MICE), a more robust but complex technique. By analyzing correlations before and after data imputation with MICE, we assessed the robustness of findings and the impact of missing data on our results. MICE is flexible and can accommodate various types of missingness and variable distributions, making it widely used in practice [15–17]. We performed multiple imputations using n=90 iterations, with a seed value of 12345, and employing the Fully Conditional Specification (FCS) method to ensure robust and reproducible results.

Analyses were performed using R version 4.3.2 and the SAS statistical suite software version 9.4 (SAS Institute Inc., Cary, NC, U.S.).

Ethical considerations

Because these analyses were for population health surveillance purposes, this study was considered non research in accordance with the Québec Public Health Act (2001, c. 60, a. 36; 2009, c. 45, a. 13). The project was reviewed by the Québec Public Health Ethics Committee and individuals were notified of the data collection and given the opportunity to opt out.

RESULTS

Baseline characteristics of participants

The study covered 714 participants with a total of 775 cases of gonococcal infection during the period between January 1, 2017, and December 31, 2019. Two cases were excluded because MIC values were missing.

The most prevalent age group was individuals over 35 years old (43.1%) and the most frequent anatomical site of infection was the urogenital site (69.1%). The majority of participants (85.0%) were men who have sex with men (MSM). Most resided in the region of Montréal (71.1%), and 33.8% of participants had five or more sexual partners in the last 2 months. Table 2 presents the baseline characteristics of participants.

Resistance / increased MIC levels

Ciprofloxacin was the antibiotic against which the highest frequency of resistance was observed (74.3%). The frequencies of resistance against tetracycline and azithromycin were respectively 19.9% and 21.3%. The proportions of increased MIC of 3GC were 9.4% for cefixime and 13.3% for ceftriaxone. Figure 1 displays the percentage of specific resistance/increased MIC levels for each antibiotic among *N. gonorrhoeae* cases in Québec, Canada, between September 1, 2017, and December 31, 2019.

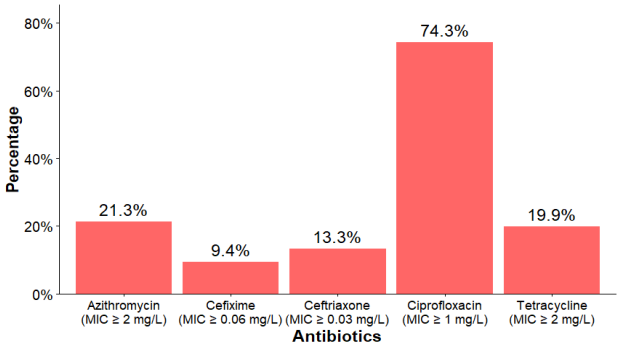

Figure 1. Specific Resistance/Increased MIC levels against each antibiotic

Table 2. Baseline characteristics of study participants between 2017 and 2019 (N=714)*

Characteristics	n	Percentage
Anatomical site of infection		
Urogenital	493	69.1%
Rectal	136	19.1%
Pharyngal	85	11.9%
Sex of sexual partners (past year)		
MSM	607	85.0%
HSM/W	107	15.0%
Age group		
Less than 25 years	131	18.4%
25 to 35 years	275	38.5%
More than 35 years	308	43.1%
Residency area		
Montréal	508	71.2%
Outside Montréal	206	28.9%
Number of sex partners (past 2 months)		
0 to 4	473	66.2%
≥ 5	241	33.8%
Material Deprivation Index		
Q1 (most privileged)	143	20.0%
Q2 to Q4	441	61.8%
Q5 (most deprived)	130	18.2%
Sex outside of Québec (past 2 months)		
Yes	137	19.2%
No	577	80.8%
Money, drugs, or other given or received in exchange for sexual relations (past year)		
Yes	114	16.0%
No	600	84.0%
Previous STBBIs		
None	231	32.4%
HIV	138	19.3%
Other than HIV	345	48.3%

MSM - men who have sex with men; HSM/W- heterosexual men and women; STBBIs-sexually transmitted and bloodborne infections; Q - quintile.
** After multiple imputation for sex of sexual partners (1.26% missing), number of sexual partners (13.3% missing), previous STBBIs (8.1% missing), sex outside of Québec (34.5% missing), material deprivation index (6.3% missing), and money, drugs, or other given or received in exchange for sexual relations (27.9% missing). There were no missing values for age, region of residence, and anatomical site of infection.*

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As summarized in Table 3, 12.5% (97/775) of *N. gonorrhoeae* isolates were classified as MDR. The most common MDR pattern, present in 4.9% of the isolates, included resistance to azithromycin, tetracycline, and ciprofloxacin, with varying susceptibility to ceftriaxone and cefixime. In contrast, 87.5% (678 isolates) were not classified as MDR, with the largest group (44.1%) showing susceptibility to azithromycin and ceftriaxone but resistance to ciprofloxacin.

Correlates of MDR gonococcal infections

The multivariate analysis using GEEs aimed to identify correlates of MDR among gonococcal infections, as detailed in Table 4. Data on all cases of gonococcal infections (n = 775) were included in the analyses.

Across analyses using both imputed and non-imputed datasets, certain factors showed consistent associations with MDR, though none of the variables demonstrated statistical significance. Examining age groups revealed interesting findings. Before imputation, the aPR was 1.65 (95% CI: 0.87-3.11, p-value: 0.118) among individuals aged 25 to 34 years and 1.28 (95% CI: 0.67-2.45, p-value: 0.453) among those aged 35 years or more. After imputation, the aPRs were 1.64 (95% CI: 0.85-3.17, p-value: 0.135) and 1.27 (95% CI: 0.65-2.49, p-value: 0.471), respectively. The number of sex partners in the past two months displayed notable associations. Before imputation, the aPR was 1.61 (95% CI: 1.07-2.41, p-value: 0.020 among individuals with five or more partners) compared to those with fewer partners. After imputation, the aPR slightly decreased to 1.52 (95% CI: 0.99-2.36, p-value: 0.054). Furthermore, other factors such as sexual orientation, having given or received money or drugs in exchange of sex exhibited aPR >1 but also did not demonstrate statistically significant associations with MDR.

Table 3. Resistance/Increased MIC to multiple antibiotics

Antibiograms						
azithromycin	**ceftriaxone (≥ 0.03 mg/L)**	**cefixime (≥ 0.06 mg/L)**	**tetracycline R**	**ciprofloxacin R[a, b]**	**n**	**Percentage**
Isolates classified as MDR					**97**	**12.5**
S	X	X	R	R	38	4.9
R			R	R	33	4.3
R	X		R	R	10	1.3
S	X		R	R	9	1.2
S		X	R	R	4	0.5
R	X	X	R	R	3	0.4
Isolates not classified as MDR					**678**	**87.5**
S			S/I	R	342	44.1
S			S/I	S/I	131	16.9
R			S/I	R	77	9.9
S			R	S/I	38	4.9
R			S/I	S/I	20	2.6
Other susceptibility profiles[c,d]					70	9.0
Total number of isolates					**775**	**100.0**

R: resistant; S: susceptible; S/I : susceptible or with intermediate susceptibility
[a] Including one isolate not susceptible to cefixime (MIC = 0.5 mg/L) and with decreased susceptibility to ceftriaxone (MIC = 0.12 mg/L);
[b] Including one isolate with decreased susceptibility to cefixime (MIC = 0.25 mg/L)
[c] Including two isolates with decreased susceptibility to cefixime (MIC = 0.25 mg/L), azithromycin S, ceftriaxone (MIC = 0.03 mg/L), tetracycline R and ciprofloxacin S/I;
[d] Isolates not classified as multidrug-resistant and n < 20.

Table 4. Multivariate analysis of correlates of multidrug resistance among gonococcal infection cases, sentinel surveillance network, Québec, Canada, 2017–2019

a-Before MICE imputation[a]						b-After MICE Imputation*					
	Characteristics	**aPR**	**95% CI**		**P-value**		**Characteristics**	**aPR**	**95% CI**		**P-value**
Age group	< 25 ans	1	-	-	-	**Age group**	< 25 ans	1	-	-	-
	25 to 34	1.65	0.87	3.11	0.118		25 to 34	1.64	0.85	3.17	0.135
	≥35	1.28	0.67	2.45	0.453		≥35	1.27	0.65	2.49	0.471
Residency area	Outside Montréal	1	-	-	-	**Residency area**	Outside Montréal	1	-	-	-
	Montréal	1.02	0.64	1.63	0.914		Montréal	0.99	0.61	1.60	0.968
Sexual orientation	HSM/F	1	-	-	-	**Sexual orientation**	HSM/F	1	-	-	-
	MSM	1.14	0.58	2.26	0.687		MSM	1.12	0.55	2.25	0.744
Number of sex partners in the past 2 months	0 - 4	1	-	-	-	**Number of sex partners in the past 2 months**	0 - 4	1	-	-	-
	≥5	1.61	1.07	2.41	0.020		≥5	1.52	0.99	2.36	0.054
	Missing	0.78	0.376	1.68	0.538						
Anatomical site of infection	Urogenital site	1	-	-	-	**Anatomical site of infection**	Urogenital site	1	-	-	-
	Pharynx	0.91	0.47	1.73	0.776		Pharynx	0.84	0.44	1.61	0.617
	Anus-rectum	0.96	0.60	1.53	0.870		Anus-rectum	0.93	0.58	1.49	0.775
Material Deprivation Index (quintiles)	Quintiles 2–4	1	-	-	-	**Material Deprivation Index (quintiles)**	Quintiles 2–4	1	-	-	-
	Q1 (most privileged)	0.92	0.56	1.51	0.749		Q1 (most privileged)	0.93	0.57	1.53	0.805
	Q5 (most deprived)	0.94	0.54	1.65	0.840		Q5 (most deprived)	0.98	0.58	1.65	0.968
	Missing	1.27	0.65	2.47	0.473						
Previous STBBIs	None	1	-	-	-	**Previous STBBIs**	None	1	-	-	-
	Other than HIV	0.97	0.55	1.71	0.930		Other than HIV	1.23	0.73	2.05	0.424
	HIV	0.98	0.46	2.16	0.950		HIV	1.02	0.50	2.09	0.938
	Missing	1.00	0.40	2.46	0.994						
Sexual relations occurred during travel outside Québec	No	1	-	-	-	**Sexual relations occurred during travel outside Québec**	No	1	-	-	-
	Yes	1.21	0.68	2.15	0.514		Yes	1.10	0.63	1.91	0.721
	Missing	1.30	0.74	2.28	0.345						
Money, drugs, or other given or received in exchange for sexual relations	No	1	-	-		**Money, drugs, or other given or received in exchange for sexual relations**	No	1	-	-	-
	Yes	1.31	0.65	2.65	0.437		Yes	1.14	0.58	2.23	0.689
	Missing	1.02	0.56	1.85	0.939						

aPR adjusted Prevalence Ratio

STBBIs sexually transmitted and bloodborne infections

[a] A specific category for missing values "missing indicator" was used when the proportion was ≥ 20%.

‡Lifetime reported STBBIs other than HIV including chlamydia, gonorrhea, syphilis, lymphogranuloma venereum, hepatitis C, hepatitis B, herpes, Trichomonas vaginalis, Mycoplasma genitalium, hepatitis A, vaginitis, scabies and crabs.

MSM - men who have sex with men; HSM/W- heterosexual men and women.

** After multiple imputation for sex of sexual partners (1.26% missing), number of sexual partners (13.3% missing), previous STBBIs (8.1% missing), sex outside of Québec (34.5% missing), material deprivation index (6.3% missing), and money,* drugs, or other given or *received in exchange for sexual relations (27.9% missing). There were no missing values for age, region of residence, and anatomical site of infection*.

DISCUSSION

In this open cohort for the surveillance of gonococcal infections in Québec, we observed a high resistance rate to ciprofloxacin (74.3%), but still low proportions of increased MIC for the 3GCs (9.4% and 13.3% respectively for cefixime and ceftriaxone). During the same study period, resistance against 3GCs was generally low across the globe: across all the 194 member states of the WHO regions, only 35% (n=69) and 26% (n=51) reported *N. gonorrhoeae* isolates with decreased susceptibility or resistance to respectively ceftriaxone and cefixime in 2017–18 [18].

The high proportion of ciprofloxacin resistance observed in our study, is consistent with global trends reflecting the increasingly challenging nature of antibiotic resistance in *N. gonorrhoeae* isolates. In fact, in Central Asia, reports showed resistance against ciprofloxacin (88.5%), ceftriaxone (12.8%) and cefixime (11.5%) [19]. A retrospective observational study of the AMR data of gonococcal isolates reported to WHO by 73 countries across the globe in 2017–18 also showed a resistance level of 77% against ciprofloxacin [18]. The antimicrobial activity of ciprofloxacin is mediated by inhibition of bacterial topoisomerases II and IV [20]. Resistance in *N. gonorrhoeae* is mainly due to mutations in the *gyrA* and/or *parC* genes, which code for these enzymes and these mutations interfere with the binding of ciprofloxacin to its target site, reducing its efficacy against bacteria [21]. The high prevalence of resistance observed against ciprofloxacin could be attributed to the emergence and rapid spread of mutant clones with mutations in these genes [21].

Our study conducted in the province of Québec shed light on the prevalence of MDR gonococcal infections, revealing a prevalence of 12.5%. This finding is consistent with national trends observed across Canada, where reports indicated fluctuating rates of MDR *N. gonorrhoeae* ranging from 12.2% in 2017 to 12.4% in 2019 [8]. Different proportions were reported in other international contexts. In Melbourne (Australia) the proportion of MDR was around 2% [22], and in Spain, the proportions ranged from 0.25% in 2016 to 0.42% in 2019 [23]. Similarly, based on their definition, England and Wales reported MDR rates at 3.5% [24]. The observed disparities in MDR prevalence highlight the complex interplay of regional factors, including healthcare practices, antimicrobial stewardship efforts, and possibly varying rates of antibiotic use and resistance patterns. Factors such as population demographics, sexual health education, accessibility to healthcare services, and surveillance systems could also contribute to the observed differences. Our findings highlighted the need for continued vigilance and collaborative efforts at local, national, and international levels to address the rising threat of MDR gonococcal infections effectively. Implementing comprehensive strategies, including enhanced surveillance, antimicrobial stewardship programs, and targeted interventions, will be crucial in curtailing the spread of MDR and safeguarding public health.

In our study, the assessment of the determinants of MDR gonococcal infections was conducted with the dataset before and after MICE imputation. The results from both sets of analyses demonstrated consistency, affirming the robustness of our findings. Even though none of them showed statistical significance, factors such as sexual exposure during travels outside of Québec as well as sexual relations given or received in exchange of money, drugs or others were found to be associated with MDR gonococcal infections in the multivariable analyses. These findings are consistent with previous research undertaken in England, Wales, and the Nordic countries of the European Union, which found similar risk factors for STIs, including gonorrhea [24–26].

Although not found significant in our analysis, it is known that transmission during travel is an important factor in the dissemination of resistant strains from one country to another. In a study published in 2017, a ceftriaxone-resistant *N. gonorrhoeae* isolate was identified in a patient in Canada, with epidemiologic and genomic data suggesting its spread from Asia [9]. Travels have been shown to be, in several studies, a risk factor of STIs in general. During travels especially for tourism purposes, people tend to have riskier sexual behaviours such as inconsistent condom use, having unprotected sex with casual partners or

engaging in sexual relations with multiple partners [27–29]. In this perspective, pre-travel consultations should be strengthened to reduce the spread of STIs, including MDR gonococcal infections. During these consultations, healthcare experts should educate tourists about sexual risks, emphasizing the importance of continuous condom use and transactional sex. Personalized advices considering the destination, activities, and health status are vital and tailored therapies are especially crucial for travelers visiting regions with high MDR gonococcal infections rates [30]. Our study also found having 5 or more sex partners in past 2 months to be associated with MDR gonorrhea. This observation could be explained by the fact that individuals having higher number of sex partners might be at higher risks of STIs leading to a more frequent use of antibiotics for treatment purposes and thereby contributing to the development and spread of MDR gonorrhea. Attempts to combat the emergence of antibiotic resistance should include not just cautious antibiotic usage, but also comprehensive initiatives that address the underlying behavioural factors of STI transmission.

While our study provided a comprehensive analysis, it is essential to acknowledge certain limitations that may affect the generalizability of our findings. The majority of participants enrolled in the sentinel network cohort were men who have sex with men (MSMs), comprising 85.0% of participants. Consequently, the applicability of our results may be restricted to populations with similar gender or sexual orientation distributions. Additionally, it is important to exercise caution in interpreting our findings as the study clinics were situated in only three 3 out of the 18 regions of the province of Québec, potentially limiting the representativeness of diverse geographical areas. Furthermore, our study relied on self-reported data, which could introduce recall and desirability biases.

CONCLUSION

Our results demonstrated an association between the number of sexual partners and MDR, reinforcing the hypothesis that MDR can develop more rapidly within dense sexual networks. The rapid development and transmission of resistant strains within these networks poses a serious threat to public health, as it facilitates the spread of dangerous and very difficult-to-treat isolates. It is also important to critically address the question of circulating resistant isolates, highlighting the need for ongoing surveillance, research and appropriate interventions to manage and contain these highly resistant strains before they become widespread.

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ETHICAL STATEMENT

Because the analyses were for population health surveillance purposes, this study was considered nonresearch in accordance with the Québec Public Health Act (2001, c. 60, a. 36; 2009, c. 45, a. 13). The Québec Public Health Ethics Committee reviewed the project, and individuals were notified of the data collection and given the opportunity to opt out.

CONFLICT OF INTEREST

None declared.

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USE OF ARTIFICIAL INTELLIGENCE TOOLS

None declared.

DATA AVAILABILITY

No additional data are available.

DISCLAIMER

The findings and conclusions in this publication are those of the authors and do not necessarily represent the official position of the authors' affiliated institutions.

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