Low prevalence of resistance genes in sheltered homeless population in Marseille, France, 2014–2018

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Objectives: The present study has explored the prevalence and potential factors contributing to the presence of nasal/pharyngeal resistant genes in homeless people.

Methods: During the winters 2014–2018, we enrolled sheltered homeless adults and controls and collected nasal/pharyngeal samples. Sixteen antibiotic resistance genes (ARGs), including genes encoding for beta-lactamases and colistin-resistance genes, were searched by real-time polymerase chain reaction (qPCR) performed directly on respiratory samples and followed by conventional PCR and sequencing.

Results: Over a 5-year period, using qPCR, we identified in homeless group (n=715) the presence of \textit{blaTEM} (396/710, 54.7%), \textit{blaSHV} (27/708, 3.6%), \textit{blaOXA-23} (1/708, 0.1%), while other genes including colistin-resistance genes (\textit{mcr}-1 to \textit{mcr}-5) were absent. We found a significantly higher proportion of ARG carriage among controls (74.1%) compared to homeless population (57.1%), \(p=0.038\). Tobacco smoking (OR=4.72, \(p=0.001\)) and respiratory clinical signs (OR=4.03, \(p=0.002\)) were most prevalent in homeless people, while vaccination against influenza (OR=0.31, \(p=0.016\)) was lower compared to controls. Among homeless people, type of housing (shelter A versus B, OR=1.59, \(p=0.006\)) and smoking tobacco (smoker versus non-smoker, OR=0.55, \(p=0.001\)) were independent factors associated with ARG carriage. By sequencing, we obtained a high diversity of \textit{blaTEM} and \textit{blaSHV} in both populations.

Conclusion: The lower risk for ARGs in the homeless population could be explained by limited access to health care and subsequently reduced exposure to antibiotics.

Keywords: antibiotic resistance gene, homeless, real-time polymerase chain reaction (qPCR), potential risk factors

Introduction

Homelessness is an increasingly social and public health concern in both developing and developed countries. Because of poor environmental conditions, poor physical state and substance abuse, this population is associated with the transmission of communicable diseases, including respiratory tract infections and multidrug-resistant tuberculosis.1,2 Antimicrobial resistance, if occurring in the homeless population, can challenge local health care systems because of the lack of surveillance due to the high mobility of this population. Several studies are available regarding the prevalence of antimicrobial resistance in homeless populations. An 8–10% prevalence of methicillin-resistant \textit{Staphylococcus aureus} was evidenced in nasal sample from homeless people in Kansas City and in Boston, USA with...
resistance to erythromycin, levofloxacin and clindamycin.3,4 A study conducted in New Orleans, USA evidenced that housing in homeless shelter was an independent risk factor for high level of penicillin-non-susceptible Streptococcus pneumoniae carriage.5

Resistance to antibiotics may be due to the production of specific inactivating enzymes, changes in membrane permeability and efflux of the antibiotic, or to alteration of target sites.6 The production of beta-lactamases (extended-spectrum beta-lactamases [ESBLs], especially those belonging to the CTX-M family, carbapenem-hydrolyzing beta-lactamases) in Gram-negative bacteria is considered the most important mechanism contributing to antimicrobial resistance to beta-lactam antibiotics.7 Due to be plasmid-borne, beta-lactamases might spread among aerobic Gram-negative bacilli including Enterobacteriaceae and non-lactose fermenting bacteria (Pseudomonas aeruginosa, Acinetobacter baumannii).7 Carbapenemases belonging to the blaOXA-23, blaOXA-24-blaOXA-48, blaOXA-58 subgroups are those most frequently identified from A. baumannii.8 Multidrug-resistant A. baumannii which has been reported a frequent cause of respiratory infection might be responsible for nosocomial infection outbreaks and lead to increase health care-associated costs and occurrence of hard-to-treat bacterial infections.9,10 Colistin is currently regarded as one of the last-resource antibiotics for a variety of treatment of human infections.11 Nevertheless, the emergence of the plasmid-mediated colistin resistance genes, such as mcr-1 gene, has also been documented in Enterobacteriaceae species and then disseminated worldwide due to the spread of a transposon, which can move in or out of plasmids and the chromosome.11

The burden of antibiotic resistance in Marseille homeless population remains unknown and unexplored. Therefore, in this cross-sectional study, we aimed to assess the prevalence of most common resistance genes carriage (genes encoding for beta-lactamases and colistin-resistance genes) in nasal-pharyngeal samples from sheltered homeless people in comparison with “non-homeless” controls. We also investigate the role of potential risk factors for resistance gene carriage. Rather than exploring antibiotic resistance by conventional culture-based methods, a molecular technique was directly applied, as this strategy has proven successful in other populations.12

Materials and methods
Selection of homeless participants
In our one-shot studies, adult homeless people residing in two municipal emergency shelters (shelters A and B) in Marseille, France were recruited on a voluntary basis in winter from 2014 through 2018. The participants were asked to complete questionnaires including information on demographics, personal history, substance use, vaccination status and respiratory clinical presentation at the time of enrolment.

Selection of comparison group
Controls (defined as the non-homeless group) included administrative staffs, physicians, nurses, medical students and Ph.D students from our institute who volunteered for nasal and pharyngeal sample collection and completed a questionnaire addressing demographics, chronic medical conditions, substance abuse, vaccination status, respiratory symptoms and signs at enrolment. This assessment was advertised only in the year 2018 and 5 days after the homeless people recruitment. Controls were selected in order to avoid marked differences in terms of age, gender or origin with the homeless group. The Méditerranée Infection Institute is at the front of the fight against health care-associated infections. The building was conceived with a specifically designed setting aiming at reducing at-risk contacts with patients.13 Staffs in clinical wards are controlled through automated continuous monitoring systems for the traceability of care and good practice reminders have been developed to an anthropological approach. In this context, the control group is unlikely to carry more resistance genes because of working at the institute.14

DNA extraction and pool
A pair of swabs (nasal and pharyngeal) were collected from each participant, transferred to Sigma-Virocult® medium and were subsequently processed for molecular analysis. The automated DNA extraction was performed on 200 µL of each swab using a BioRobot®EZ1 Advanced XL instrument (QIAGEN, Hilden, Germany) and DNeasy® Blood & Tissue according to the manufacturer’s instructions. DNA pooling was performed as previously described.15 The DNA extraction quality of each pool was assessed by RT-PCR targeting internal control TISS phage that was added to each extraction.16

Real-time PCR
All quantitative real-time PCR (qPCR) reactions were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, USA) with the ready-to-use reaction mix ROX qPCR Master according to the manufacturer’s recommendations. Negative control (single PCR mix and sterile H2O) and positive control template (Plasmid DNA extracted from
consequences of 1) most common β-lactamase encoding genes including blaTEM, blaSHV, ESBL genes (blaCTX-A and blaCTX-B [blaCTX-M clusters A and B]) and carbapenemase-encoding genes (blaOXA-23, blaOXA-24, blaOXA-48, blaOXA-58, blaNDM, blaVIM, blaKPC), and 2) colistin-resistance genes (mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5) by using primers as described and by using specific primers designed in our laboratory (Table 1).

Conventional PCR and sequencing

To better characterize these genes, only positive qPCR results were simultaneously tested by standard PCR. The purified PCR products were sequenced using specific primers and the BigDye Terminator® version 1.1 cycle sequencing ready reaction mix (Applied Biosystems, Foster City, CA, USA). All primers used in this study have previously been described (Table 1). Sequencing was performed on Applied Biosystems 3130 platform (ABI PRISM, PE Applied Biosystems, USA). Obtained sequences were edited and assembled using Chromas Pro 1.77 (Technelysium Pty Ltd, Australia) and were then aligned with reference genes from the ARG-ANNOT by Mega 7.0 software (https://www.megasoftware.net).

Statistical analysis

Collected data were statistically treated using Microsoft Excel 2016 and STATA (version 11.1). Missing data and unidentified samples were not analyzed. Statistical differences in baseline characteristics were evaluated by Pearson’s chi-square or Fisher’s exact tests as categorical variables. Means of quantitative data were compared using Student’s t-test. A two-tailed p-value <0.05 was considered as statistically significant. We first compared the prevalence of at least one resistance gene between the homeless group and the control group (in the year 2018). In addition, two logistic regression models were applied in order 1) to compare the distribution of potential risk factors among homeless and control groups (in the year 2018); 2) to identify factors associated with resistance gene carriage in the homeless group (in the 2014–2018 period). In the first model, univariate analysis was used to examine unadjusted distributions of multiple factors (demographic, chronic medical condition, respiratory symptoms or physical finding between. A p-value <0.05 was considered statistically significant. Variables with p-values of <0.2 from the univariate analysis were included in the multivariable multinomial regression, which was then created by step-wise regression. Using the same methods, in the second model, the same set of potential risk factors together with special characteristic for homeless (type of housing, duration of homelessness) were considered for association with resistance genes. Variation over time was adequate (assessed statistically as the proportion of variance explained by year and considered adequately fitted if the coefficient of determination [R² statistic] was >50%) by using Microsoft Excel 2016.

Results

A total of 724 homeless people was included in the study, of whom 715 (98.8%) had a nasal and/or pharyngeal swab. In the comparison group, 54 individuals were recruited and all provided both nasal and pharyngeal swabs. A total of 1,530 respiratory samples was collected. The DNA was grouped into 87 pools (15 pools of 10, 12 pools of 15 and 60 pools of 20).

Participant characteristics

The homeless individuals were predominantly male (98.2%) with a median age of 43 years (ranging 18–84 years). Fifty-five percent of them were recruited from shelter A and 45% from shelter B. Participants originated from 49 countries with a majority of African migrants (70%). About 54% of the migrant population arrived in France more than a year earlier, with a mean (SD) length of stay in France of 10 years. Overall, the average duration of homelessness was about 3 years. A proportion of 9.6% of homeless people reported chronic respiratory disease and 60.3% smoking tobacco. About 40% were suffering from at least one respiratory symptom or sign at the time of sampling, with a 27.4% cough prevalence. Vaccination rate against influenza was less than 15%. Other socio-demographic characteristics, substance abuse, chronic diseases and clinical features of participants are shown in Table 2.
| Gene | Name | Primers (5′-3′) and probes | Amplicon size (pair of base) | Reference |
|------|------|-----------------------------|----------------------------|-----------|
| A. Real-time PCRs | blaTEM | Forward TTTCTGCTATGTGGTGGTTCGGA TTCTTGGCTAGTGTTGATG | 213 | (15) |
| | | Reward GTCTCGATGTGGTGGTTCGGA | | |
| | | Probe 6-FAM-AACTCGGAGTCAGACAGCTTCCG-TAMRA | | |
| | blaSHV | Forward TCCCATGATGACCACCTTTAAA | 105 | (15) |
| | | Reward TCTGCTGGCAGTATGTTGATG | | |
| | | Probe 6-FAM-TGCGACGAGTACAGCCTACCG-TAMRA | | |
| | blaCTX-M-A | Forward CGGCRATGCGGCAACAC | 105 | (15) |
| | | Reward TGCRCCTCGGATATTGCC | | |
| | | Probe 6-FAM-CCACGCGGAGCAGCCG-TAMRA | | |
| | blaCTX-M-B | Forward ACCGACCCAGCCTTA | 221 | (15) |
| | | Reward 6-FAM-CCGAGCCGAGATACCAACCGC-TAMRA | | |
| | blaKPC | Forward GATACCGTGGCTCGTTGGA | 180 | (16) |
| | | Reward GTCTGCTTTTGTACATG | | |
| | | Probe 6-FAM-CGCGACGAGTACAGCCTACCG-TAMRA | | |
| | blaNDM | Forward GCGCAACACAGCCTTCTT | 155 | (16) |
| | | Reward CAGCACAAAGACCGATGC | | |
| | | Probe 6-FAM-CAACACGCGCCCAAACCTTGGC-TAMRA | | |
| | BlaVIM | Forward CACAGYGGCCCTTTCTGGAG | 132 | (16) |
| | | Reward GCGTAAGGTGGCCCTTCGGCAAC | | |
| | | Probe 6FAM-A | | |
| | | mcr-1–2 | Forward TGCTCAAAGGCCGCAATA | 130 | (16) |
| | | Reward TACCCCTTTCGCGCCTTCC | | |
| | | Probe 6-FAM-GGTTGAGGCAATGCGGACTTCA-TAMRA | | |
| | blaOXA-23 | Forward CAAATGGAGGTTTCTTGGGGAATA | 123 | (16) |
| | | Reward TCCGCTTGCTACGTCCCTTGAT | | |
| | | Probe 6-FAM-GGTTGAGGCAATGCGGACTTCA-TAMRA | | |
| | blaOXA-24 | Forward TCTTAACCGGGCAACCAT | 125 | (16) |
| | | Reward GCGTCTGGCTGCCATCCACATT | | |
| | | Probe 6-FAM-GGTTGAGGCAATGCGGACTTCA-TAMRA | | |
| | blaOXA-48 | Forward TGCTCAAAGGCCGCAATA | 102 | (16) |
| | | Reward TCCGCTTGCTACGTCCCTTGAT | | |
| | | Probe 6-FAM-GGTTGAGGCAATGCGGACTTCA-TAMRA | | |
| | blaOXA-58 | Forward TACCCCTTTCGCGCCTTCC | 125 | (16) |
| | | Reward TCCGCTTGCTACGTCCCTTGAT | | |
| | | Probe 6-FAM-GGTTGAGGCAATGCGGACTTCA-TAMRA | | |
| | mcr-1–2 | Forward CTGTCCCATGTATGTTGATTCC | 151 | Available in our laboratory |
| | | Reward TTATCCATCAGCGCTTCCTTA | | |
| | | Probe (mcr-1–2) FAM-TATGATGCAGACCGGCTTTTGGGGAATA-TAMRA | | |
| | | Probe (mcr-2) VIC-TATGATGCAGACCGGCTTTTGGGGAATA-TAMRA | | |
| | mcr-3 | Forward TGGAATTCTACGCACTTCAG | 144 | Available in our laboratory |
| | | Reward TGCTGCAACACCCGCTACATCAAC | | |
| | | Probe FAM-TGACCGGGAATGCAGCAGCCGGTG-TAMRA | | |
| | mcr-4 | Forward GCCAACCACGATCCGATACCCGCAATA | 112 | Available in our laboratory |
| | | Reward CCGCCCATCCTTGGAAAAACATAC | | |
| | | Probe FAM-GGACCGGAGGTTTGGCTTACCC-TAMRA | | |

(Continued)
Controls were significantly more likely to be vaccinated against influenza compared to homeless people enrolled in 2018. Homeless people were significantly more likely to report chronic diseases and tobacco smoking and to present with respiratory symptoms at sampling time, compared to controls (Table 3). The differences in the distribution of several factors between the two groups remained significant in multivariate multinomial regression, such as tobacco smoking, respiratory clinical signs and vaccination against influenza (Table 4).

Screening of β-lactamase encoding genes (Table 5 and Figure 1)

**blaTEM**

Among the pools tested by qPCR screening, 82.8% (72/87) were positive for *blaTEM*. By individual retesting of each specimen for positive pools, we found a prevalence of 54.7% (396/710) in the homeless group over a 5-year period. Interestingly, in the control group, the prevalence was of 72.2% in 2018 (39/54), which was significantly higher than in the homeless group recruited in 2018 (52%, 51 of 98, \( p < 0.001 \)). Of note, using sequencing reaction targeting *blaTEM* in the 72 pools testing positive for *blaTEM*, we succeeded in amplifying 69 sequences and obtained 5 sequence types from the homeless group and 2 from the comparison group, showing 99–100% nucleotide identity to *blaTEM* type/reference genes in ARG-ANNOT site (Figure 1A).

**blaSHV**

About 18.4% (16/87) of the pools were positive for *blaSHV*-qPCR. In the homeless group, *blaSHV* prevalence was 3.8% (27/708). There was no significant difference of prevalence between the two groups in 2018 (7/98 in homeless people versus 4/54 in controls, \( p = 0.92 \)).

For the genotypic identification of *blaSHV*, we succeeded in amplifying 25 sequences (of 27, 92.6%) for homeless population and 4 (of 4, 100%) for the comparison group. The sequence results show the diversity of *blaSHV* in the homeless group (comprising 11 sequence types) and in the control group (comprising 3 sequence types) (Figure 1B).

**blaCTX-M-A and blaCTX-M-B**

None of the DNA pools tested positive for *blaCTX-M-A* and *blaCTX-M-B*.

**Carbapenemase-encoding genes**

Only 1 homeless (0.14%) was positive for *blaOXA-23* by both qPCR and sequencing in nasal swab sampled in 2014 (Figure 1C). None of the DNA pools tested positive for *blaOXA-58*, *blaOXA-48*, *blaOXA-24*, *blaNDM*, *blaVIM*.

**Screening of colistin-resistance genes**

None of the DNA pools tested positive for *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* genes.

Overall, by qPCR, 57.5% (408/710) samples were positive for at least one resistance gene (*blaSHV* or *blaTEM* or *blaOXA-23*) in the homeless group; the proportion of *blaSHV* (or *blaTEM*) genes did not significantly vary over time (Figure 2). A higher prevalence of at least one resistance gene was observed in the comparison group (40 of 54, 74.1%) compared to the homeless group (56 of 98, 57.1%, \( p = 0.038 \)) (Figure 3).

### Table 1 (Continued).

| Gene     | Name       | Primers (5′-3′) and probes                        | Amplicon size (pair of base) | Reference                   |
|----------|------------|--------------------------------------------------|------------------------------|-----------------------------|
| mcr-5    | Forward    | TATCCCGCAAGCTACCGACGC                            | 126                          | Available in our laboratory  |
| Reward   |            | ACGGGCAAGCACATGATCGGT                            |                              |                             |
| Probe    |            | FAM-TGGCAACCACCGATCTGGCCA-TAMRA                  |                              |                             |

B. Conventional PCRs

| Gene   | Forward | Amplicon size (pair of base) | Reference |
|--------|---------|------------------------------|-----------|
| *blaTEM* | ATGAGTATTCAACATTTCCGTG      | 861            | (15)      |
|         | TACCAATGCTATACTGAGG         |               |           |
| *blaSHV* | ATTTGTCGCTTCTTACTGCG       | 1051           | (15)      |
|         | TTTATGGCGTTCCTTTGACC        |               |           |
| *blaOXA-23* | GATCCGATTGGAAGACGAGA        | 501            | (16)      |
|         | ATTTCTGACCCGATTCCAT         |               |           |
Factor associated with resistance genes prevalence in the homeless population: multivariate model

Resistance genes prevalence was significantly higher in participants housed in shelter A (compared to shelter B) and in those born in African or Asian countries compared to European countries. Individuals smoking tobacco and cannabis were less likely to present resistance genes than others. In multivariate analysis, only being housed in shelter A (OR=1.59 [1.14–2.2], p=0.006) and smoking tobacco...
Table 2 Characteristics of homeless participants: Demographics, chronic medical conditions and respiratory for resistance gene carriage (N=724 individuals)

| Characteristics          | Total n (%) | At least one resistance gene n (%) | No resistance gene n (%) | Univariate analysis OR (95% CI), p-value |
|--------------------------|-------------|------------------------------------|--------------------------|----------------------------------------|
| Total                    |             | 408 (57.5)                         | 302 (42.5)               |                                        |
| **Year of study**        |             |                                    |                          |                                        |
| 2014                     | 144 (19.9)  | 63 (45.7)                          | 75 (54.3)                | -                                      |
| 2015                     | 126 (17.4)  | 90 (72.6)                          | 34 (27.4)                | -                                      |
| 2016                     | 157 (21.7)  | 86 (55.5)                          | 69 (44.5)                | -                                      |
| 2017                     | 198 (41.3)  | 113 (57.9)                         | 82 (42.1)                | -                                      |
| 2018                     | 99 (13.7)   | 56 (57.1)                          | 42 (42.9)                | -                                      |
| **Shelter**              |             |                                    |                          |                                        |
| B                        | 323 (44.6)  | 164 (51.4)                         | 155 (48.6)               | REF                                    |
| A                        | 401 (55.4)  | 244 (62.4)                         | 147 (37.6)               | 1.57 (1.16–2.19), p=0.003              |
| **Gender**               |             |                                    |                          |                                        |
| Female                   | 13 (1.8)    | 3 (23.1)                           | 9 (76.9)                 | -                                      |
| Male                     | 705 (98.2)  | 401 (58.0)                         | 290 (42.0)               | -                                      |
| Unknown                  | 6 (-)       |                                    |                          |                                        |
| **Age at enrolment**     |             |                                    |                          |                                        |
| Mean age (SD)            | 42.8±16 years | N/A                             | N/A                      |                                        |
| Age range (years)        |             |                                    |                          |                                        |
| ≤30                      | 186 (26.0)  | 114 (57)                           | 86 (43)                  | REF                                    |
| 30–≤50                   | 286 (39.9)  | 157 (59.5)                         | 124 (44.1)               | 0.96 (0.66–1.38), p=0.81               |
| >50                      | 244 (34.1)  | 133 (59.9)                         | 89 (40.1)                | 1.12 (0.77–1.66), p=0.55               |
| Unknown                  | 8 (-)       |                                    |                          |                                        |
| **Origin**               |             |                                    |                          |                                        |
| European                 | 182 (25.3)  | 85 (48.0)                          | 92 (52.0)                | REF                                    |
| African                  | 502 (69.8)  | 299 (60.4)                         | 196 (39.6)               | 1.65 (1.17–2.33), p=0.004              |
| Asian                    | 35 (4.9)    | 23 (67.6)                          | 11 (32.4)                | 2.27 (1.05–4.92), p=0.036              |
| Unknown                  | 6 (-)       |                                    |                          |                                        |
| Mean duration of residence in France for migrant (SD), range (min, max) (years) | 9.6±15.8 (0–66) | N/A                             | N/A                      |                                        |
| Mean duration of homelessness (SD) | 2.8±5.5 | N/A                             | N/A                      |                                        |
| Range of duration of homelessness (years) |            |                                    |                          |                                        |
| <1 year                  | 371 (53.2)  | 216 (59.7)                         | 146 (40.3)               | REF                                    |
| ≥1 year                  | 327 (46.8)  | 179 (55.2)                         | 145 (44.8)               | 0.83 (0.61–1.13), p=0.25               |
| Unknown                  | 25 (-)      |                                    |                          |                                        |
| **Alcohol**              |             |                                    |                          |                                        |
| Frequent                 | 82 (11.5)   | 39 (49.4)                          | 40 (50.6)                | 0.69 (0.43–1.1), p=0.12                |
| Rare or never            | 633 (88.5)  | 366 (58.7)                         | 258 (41.3)               | REF                                    |
| Unknown                  | 9 (-)       |                                    |                          |                                        |
| **Tobacco**              |             |                                    |                          |                                        |
| Never                    | 284 (39.7)  | 188 (67.1)                         | 92 (32.9)                | REF                                    |
| Yes                      | 432 (60.3)  | 217 (51.3)                         | 206 (48.7)               | 0.51 (0.38–0.71), p<0.0001             |

(Continued)
remained associated with resistance gene carriage (Table 6).

Discussion

A report from the Public Assistance – Hospitals of Marseille estimated that there are approximately 1,500 homeless individuals, including 800 sleeping on city streets, park benches, and subway trains and approximately 600 residing temporarily at the 2 main municipal shelters, with a high turnover. This is the first retrospective study aiming to assess directly resistance gene carriage rates in nasal/pharyngeal samples among sheltered homeless and their potential risk factors. As mentioned in several studies, tobacco smoking and suffering of respiratory symptoms at inclusion were strongly associated with homelessness status. By contrast, the prevalence of seasonal vaccination against influenza was lower in the homeless group compared to controls; in fact, the seasonal vaccination coverage in homeless people ≥65 years was only 32.9% compared to 48.5–50.8% in the overall general French population ≥65 years in the same period.

Homeless people from shelter A were more likely to carry resistance genes compared to those from shelter B. A sub-population of homeless people with a high level of precariousness is housed in a special sector of

Table 2 (Continued).

| Characteristics                        | Total n (%) | At least one resistance gene n (%) | No resistance gene n (%) | Univariate analysis OR (95% CI), p-value |
|----------------------------------------|-------------|-----------------------------------|--------------------------|----------------------------------------|
| Unknownb                               | 9 (-)       | 55 (45.1)                         | 67 (54.9)                | 0.54 (0.37–0.8), p=0.002               |
| Cannabis                               | 126 (17.6)  | 1 (50)                            | 1 (50)                   | -                                      |
| Intravenous drug use                   | 3 (0.4)     | 10 (47.6)                         | 11 (52.4)                | -                                      |
| Snorted drug use                       | 22 (3.1)    | 3 (33.3)                          | 6 (66.7)                 | -                                      |
| Drug substitutes                       | 9 (1.3)     |                                   |                          |                                        |
| Chronic diseases                       |             |                                   |                          |                                        |
| Chronic respiratory diseasesc          | 69 (9.6)    | 32 (47.8)                         | 35 (52.2)                | 0.65 (0.4–1.09), p=0.1                 |
| Diabetes mellitus                      | 47 (6.5)    | 27 (57.4)                         | 20 (42.6)                | 1.01 (0.56–1.54), p=0.97              |
| Cancer                                 | 6 (1.0)     | 4 (66.7)                          | 2 (33.3)                 | -                                      |
| Hepatitis                              | 16 (2.8)    | 6 (37.5)                          | 10 (62.5)                | -                                      |
| BMI                                    |             |                                   |                          |                                        |
| Mean BMI (kg/m2)                       | 24.2±4.1    | N/A                               | N/A                      |                                        |
| Range of BMI (kg/m2)                   | 14.7–40.1   | N/A                               | N/A                      |                                        |
| Normal weight                          | 393 (57.8)  | 223 (57.9)                        | 162 (42.1)               | REF                                    |
| Underweight                            | 31 (4.6)    | 17 (54.8)                         | 14 (42.2)                | 0.88 (0.42–1.81), p=0.74              |
| Overweight                             | 194 (28.6)  | 103 (53.1)                        | 91 (46.9)                | 0.82 (0.58–1.16), p=0.27              |
| Obesity                                | 61 (9.0)    | 37 (61.7)                         | 23 (38.3)                | 1.17 (0.67–2.04), p=0.58              |
| Unknownb                               | 45 (-)      |                                   |                          |                                        |
| Seasonal vaccination against influenzad| 103 (14.6)  | 52 (51.5)                         | 49 (48.5)                | 0.76 (0.5–0.156), p=0.197             |
| Clinical findings                      |             |                                   |                          |                                        |
| At least one respiratory symptom or sign | 266 (39.5) | 149 (57.5)                        | 110 (42.5)               | 0.96 (0.7–1.32), p=0.81               |
| Cough                                  | 182 (27.4)  | 101 (56.7)                        | 77 (43.3)                | 0.91 (0.64–1.29), p=0.58              |
| Expectoration                          | 83 (12.7)   | 42 (51.2)                         | 40 (48.8)                | 0.71 (0.45–1.3), p=0.145             |
| Rhinorrhea                             | 49 (10.9)   | 28 (58.3)                         | 20 (41.7)                | 1.1 (0.6–1.99), p=0.8                 |
| Fever                                  | 14 (2.5)    | 7 (50)                            | 7 (50)                   |                                        |

The variable was not included in the analysis, given that no intervention could be done based on this criterion

Unknown: missing data or unidentified samples

Chronic respiratory diseases were defined as suffering from one of the following conditions: asthma, chronic obstructive pulmonary disease, occupational lung diseases and pulmonary hypertension

Proportion of seasonal vaccination against influenza for individuals ≥65 years of age: 24 of 73 participants (32.9%)

At least one respiratory symptom and sign was defined as suffering from one of the following coughs, expectoration, rhinorrhea, dyspnea, sore throat, sibilants, rhonchi, crackles

Abbreviations: BMI, body mass index; NA, not applicable; REF, reference category.

(OR=0.55 [0.39–0.78], p=0.001) remained associated with resistance gene carriage (Table 6).
shelter A, which may partially explain our results. Unfortunately, the fact of being housed in the special unit was not documented on a regular basis in our surveys. We also found a negative association between tobacco smoking and resistance gene carriage. Tobacco has been shown to have impact on the nasopharyngeal flora of smokers, which may possibly account for the lower prevalence of resistance gene in homeless smokers, although further studies are needed before conclusions can be drawn.

Overall, a lower proportion of \(\beta\)-lactamase encoding gene carriage was observed among the homeless individuals compared to controls. A possible explanation is the limited access to health care and subsequently reduced exposure to

### Table 3 Comparison of homeless people and controls (year 2018)

| Characteristics                  | Homeless group n (%) | Control group n (%) | Univariate analysis OR (95% CI) | p-value |
|----------------------------------|----------------------|---------------------|---------------------------------|---------|
| Total                            | 99                   | 54                  | N/A                             | 1.00    |
| Genre                            |                      |                     |                                 |         |
| Male                             | 99 (100)             | 54 (100)            | N/A                             | 1.00    |
| Female                           | 0                    | 0                   | N/A                             | 0.06    |
| Mean age (SD)                    | 39.4±17.5            | 34.4±10.2           |                                 |         |
| Age range                        |                      |                     |                                 |         |
| ≤30 years of age                 | 47 (47.5)            | 27 (50.0)           | REF                             | 0.67    |
| 30-550 years of age              | 24 (24.2)            | 20 (37.0)           | 0.67 (0.32–1.47)                | 0.34    |
| >50 years of age                 | 28 (28.3)            | 7 (13.0)            | 2.30 (0.88–5.96)                | 0.09    |
| Origin                           |                      |                     |                                 |         |
| Europe                           | 22 (22.2)            | 19 (35.2)           | REF                             | 2.28    |
| Africa                           | 74 (72.6)            | 28 (27.5)           | 0.27 (0.08–1.63)                | 0.19    |
| Asia                             | 3 (30.0)             | 7 (70.0)            | N/A                             | 0.117   |
| Mean duration of residence in France (SD) for migrants (min, max) | 8.2±16.1 (1 week-63 years) | 3.8±3.9 (5 months-17 years) |                                 |         |
| Addiction                        |                      |                     |                                 |         |
| Tobacco consumption              | 58 (59.2)            | 13 (24.1)           | 4.60 (2.20–9.60)                | <0.0001 |
| Alcohol                          | 13 (13.3)            | 6 (11.1)            | 1.22 (0.43–3.43)                | 0.7     |
| Antibiotic use in past 2 weeks   | 5 (5.2)              | 5 (9.3)             | 1.90 (0.68–4.51)                | 0.27    |
| Chronic respiratory diseases     | 10 (10.1)            | 0 (0)               | N/A                             | <0.0001 |
| Seasonal vaccination against influenza | 13 (13.4)       | 17 (31.5)           | 0.33 (0.15–0.76)                | 0.008   |
| Vaccination against pneumococcus | 3 (3.2)              | 4 (7.4)             | 1.39 (0.34–4.00)                | 0.7     |
| Clinical findings                |                      |                     |                                 |         |
| At least one respiratory symptom and sign | 41 (41.8)    | 8 (14.8)            | 4.67 (2.00–10.96)               | 0.001   |
| Fever (temperature measured)     | 0 (0)                | 0 (0)               | N/A                             | N/A     |

Abbreviations: NA, not applicable; Ref, reference category.

### Table 4 Multivariate analysis of distribution of demographics, chronic medical conditions, clinical finding between the two groups (homeless people versus controls)

| Characteristics* | Multivariate analysis OR (95% CI), p-value |
|------------------|-------------------------------------------|
| Origin           |                                           |
| Tobacco consumption | 4.72 (2.12–10.53), p<0.0001  |
| Seasonal vaccination against influenza | 0.31 (0.12–0.81), p=0.016  |
| At least one respiratory symptom and sign | 4.03 (1.64–9.90), p=0.002  |

*Only variables with p-values of <0.2 in the univariate analysis
antibiotics in homeless people, since, in France, antibiotics are not available for sale without prescription. Further longitudinal studies are needed to better assess the antibiotic use in this population and to challenge this hypothesis.

Table 5 Prevalence of antibiotic resistance genes in nasal/pharyngeal samples in homeless population in the period 2014–2018 (N=715 individuals)

| Overall gene frequency | N (%) |
|-----------------------|-------|
| At least one resistance gene | 408 (57.5) |
| Extended-spectrum beta-lactamases | 407/710 (57.3) |
| blaTEM | 396/710 (54.7) |
| blaSHV | 27/708 (3.8) |
| blaCTX-M-A | 0 |
| blaCTX-M-B | 0 |
| Carbapenemase encoding-genes | 1/708 (0.14) |
| blaOXA-23 | 1/708 (0.14) |
| blaOXA-24 | 0 |
| blaOXA-48 | 0 |
| blaOXA-58 | 0 |
| blaKPC | 0 |
| blaVIM | 0 |
| blaNDM | 0 |
| Colistin genes | |
| mcr-1 | 0 |
| mcr-2 | 0 |
| mcr-3 | 0 |
| mcr-4 | 0 |
| mcr-5 | 0 |

To date, more than 400 members of blaTEM and blaSHV have been described. It is proven that blaTEM-1 is one of the major plasmids associated with H. influenzae which is a major causative bacterium of community-acquired respiratory tract infections. In a previous work, a high prevalence of Haemophilus influenzae (59%) was shown in this population during the period 2015–2017. In this study, similarly high genetic diversity among blaTEM-encoding strains was observed; each blaTEM has one (blaTEM-2, blaTEM-29, blaTEM-55, blaTEM-59, blaTEM-96, blaTEM-171) amino acid substitution when compared to blaTEM-1. For blaSHV-1, a non-ESBL variant was first identified as plasmid-encoded in E. coli from Switzerland and subsequently described worldwide in isolates in different epidemiological settings, both in humans and animals. The genetic diversity of blaSHV-encoding gene in our study did not correlate with the origin of migrants (data not shown).

We reported a very low prevalence of carbapenemase-encoding genes (only blaOXA-23 with 1 of 708 individuals, 0.14%). In surveys conducted among healthy French pilgrims before departure to the Hajj pilgrimage, the prevalence of carbapenemase-encoding was also low (1% blaOXA-48) when detected by the same molecular method.

Since the first description of mobilized colistin gene mcr-1 in a plasmid carried by E. coli isolated in China in April 2011, the dissemination of the transposon has been reported in numerous countries across five continents. Few data are available on the prevalence of mcr-genes.
other than mcr-1 in the human sample. In France, to our knowledge, mcr-genes others than mcr-1 have rarely been reported,33–37 and no data is available concerning the presence of mcr-2 to mcr-5 in human samples.

Our study was based on non-culture techniques for screening antimicrobial resistance. Because many resistance genes are located on plasmids, resistance screening by direct qPCR provides a quick and simple method of screening products from genetic manipulations and a rapid estimation of antimicrobial resistance. Furthermore, pooling DNA reduces the cost of real-time PCR and yields a high specificity and a high sensitivity.16

Our work has several limitations. Homeless population was not randomly selected so that those harboring respiratory symptoms (cough, expectoration, rhinorrhoea) might have been more prone to enroll in the survey given that a medical examination was offered. The information about antibiotic use in the past, covered only a short 2-week period, which does not allow evaluating a possible lower exposure to antibiotics in the homeless group compared to controls. The detection of resistance gene directly from specimens did not allow to identify the bacteria that housed the antibiotic resistance genes.

**Conclusions**

Notwithstanding these limitations, the current study evidenced an unexpected low rate of resistance gene carriage among homeless people that could be explained by limited access to health care and subsequently reduced exposure to antibiotics.

**Ethics approval and informed consent**

This protocol was reviewed and approved by the Marseille Institutional Review Board/Ethics Committee (Homeless population: 2010-A01406-33; Comparison group: 07-008-IFR 48). Informed consent was dated and signed by all
individuals and the study was conducted in accordance with the Declaration of Helsinki.

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Author contributions
All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure
The authors report no conflicts of interest in this work.

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