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ORIGINAL RESEARCH

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 bla_{TEM} (396/710, 54.7%), bla_{SHV} (27/708, 3.6%), $bla_{\text{OXA-23}}$ (1/708, 0.1%), while other genes including colistin-resistance genes (*mcr*-1 to *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<0.0001) 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 bla_{TEM} and bla_{SHV} 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 *Staphylococcus aureus* was evidenced in nasal sample from homeless people in Kansas City and in Boston, USA with

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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 β-lactamases [ESBLs], especially those belonging to the CTX-M family, carbapenem-hydrolyzing β-lactamases) in Gram-negative bacteria is considered the most important mechanism contributing to antimicrobial resistance to β-lactam antibiotics.[7] Due to be plasmid-borne, β-lactamases might spread among aerobic Gram-negative bacilli including *Enterobacteriaceae* and non-lactose fermenting bacteria (*Pseudomonas aeruginosa, Acinetobacter baumannii*).[7] Carbapenemases belonging to the $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-48}}$, $bla_{\text{OXA-58}}$ 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 β-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 H_2O) and positive control template (Plasmid DNA extracted from

a colony of *Escherichia coli* or *K. pneumoniae* cultured) were included in each qPCR experimental run. Results were considered positive accepted when the cycle threshold value of real-time PCR was ≤35. Individual retesting of each specimen was carried out from positive pools. Eventually, individuals having at least a nasal or a pharyngeal positive sample were considered positive cases.

The qPCR amplification was used to confirm the presence of 1) most common β-lactamase encoding genes including bla_{TEM}, bla_{SHV}, ESBL genes (bla_{CTX-A} and bla_{CTX-B} [bla_{CTX-M} clusters A and B]) and carbapenemase-encoding genes (bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48}, bla_{OXA-58}, bla_{NDM}, bla_{VIM}, bla_{KPC}), 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).[17,18]

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).[17,18] 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).[19] These sequences are available in GenBank at accession numbers from MK099071 to MK099084 (for bla_{SHV}), from MK099085 to MK099091 (for bla_{TEM}) and MK099092 (for bla_{OXA-23}) (Figure 1).

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 [R^2 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.

Table 1 Sequences of primers and probes used for real-time PCRs and conventional PCRs in this study

Gene	Name	Primers (5′-3′) and probes	Amplicon size (pair of base)	Reference
A. Real-time PCRs				
bla_{TEM}	Forward	TTCTGCTATGTGGTGCGGTA	213	(15)
	Reward	GTCCTCCGATCGTTGTCAGA		
	Probe	6-FAM-AACTCGGTCGCCGCATACACTATTCTCAGA-TAMRA		
bla_{SHV}	Forward	TCCCATGATGAGCACCTTTAAA	105	(15)
	Reward	TCCTGCTGGCGATAGTGGAT		
	Probe	6-FAM-TGCCGGTGACGAACAGCTGGAG-TAMRA		
$bla_{CTX-M-A}$	Forward	CGGGCRATGGCGCARAC	105	(15)
	Reward	TGCRCCGGTSGTATTGCC		
	Probe	6-FAM-CCARCGGGCGCAGYTGGTGAC-TAMRA		
$bla_{CTX-M-B}$	Forward	ACCGAGCCSACGCTCAA	221	(15)
	Reward	CCGCTGCCGGTTTTATC		
	Probe	6-FAM-CCCGCGYGATACCACCACGC-TAMRA		
bla_{KPC}	Forward	GATACCACGTTCCGTCTGGA	180	(16)
	Reward	GGTCGTGTTTCCCTTTAGCC		
	Probe	6-FAM-CGCGCGCCGTGACGGAAAGC-TAMRA		
bla_{NDM}	Forward	GCGCAACACAGCCTGACTTT	155	(16)
	Reward	CAGCCACCAAAAGCGATGTC		
	Probe	6-FAM-CAACCGCGCCCAACTTTGGC-TAMRA		
Bla_{VIM}	Forward	CACAGYGGCMCTTCTCGCGGAGA	132	(16)
	Reward	GCGTACGTYGCCACYCCAGCC		
	Probe	6FAM-AGTCTCCACGCACTTTCATGACGACCGCGTCGGCG-TAMRA		
bla_{OXA-23}	Forward	TGCTCTAAGCCGCGCAAATA	130	(16)
	Reward	TGACCTTTTCTCGCCCTTCC		
	Probe	6-FAM-GCCCTGATCGGATTGGAGAACCA-TAMRA		
bla_{OXA-24}	Forward	CAAATGAGATTTTCAAATGGGATGG	123	(16)
	Reward	TCCGTCTTGCAAGCTCTTGAT		
	Probe	6-FAM-GGTGAGGCAATGGCATTGTCAGCA-TAMRA		
bla_{OXA-48}	Forward	TCTTAAACGGGCGAACCAAG	125	(16)
	Reward	GCGTCTGTCCATCCCACTTA		
	Probe	6-FAM-AGCTTGATCGCCCTCGATTTGG-TAMRA		
bla_{OXA-58}	Forward	CGCAGAGGGGAGAATCGTCT	102	(16)
	Reward	TTGCCCATCTGCCTTTTCAA		
	Probe	6-FAM-GGGGAATGGCTGTAGACCCGC-TAMRA		
mcr-1–2	Forward	CTGTGCCGTGTATGTTCAGC	151	Available in our laboratory
	Reward	TTATCCATCACGCCTTTTGAG		
	Probe (*mcr*-1–2)	FAM-TATGATGTCGATACCGCCAAATACC-TAMRA		
	Probe (*mcr*-2)	VIC-TGACCGCTTGGGTGTGGGTA-TAMRA		
mcr-3	Forward	TGAATCACTGGGAGCATTAGGGC	144	Available in our laboratory
	Reward	TGCTGCAAACACGCCATATCAAC		
	Probe	FAM-TGCACCGGATGATCAGACCCGT-TAMRA		
mcr-4	Forward	GCCAACCAATGCTCATACCCAAAA	112	Available in our laboratory
	Reward	CCGCCCCATTCGTGAAAACATAC		
	Probe	FAM-GCCACGGCGGTGTCTCTACCC-TAMRA		

(*Continued*)

Table 1 (Continued).

Gene	Name	Primers (5′-3′) and probes	Amplicon size (pair of base)	Reference
mcr-5	Forward Reward Probe	TATCCCGCAAGCTACCGACGC ACGGGCAAGCACATGATCGGT FAM-TGCGACACCACCGATCTGGCCA-TAMRA	126	Available in our laboratory
B. Conventional PCRs				
bla_{TEM}	Forward Reward	ATGAGTATTCAACATTTCCGTG TTACCAATGCTTAATCAGTGAG	861	(15)
bla_{SHV}	Forward Reward	ATTTGTCGCTTCTTTACTCGC TTTATGGCGTTACCTTTGACC	1051	(15)
bla_{OXA-23}	Forward Reward	GATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCAT	501	(16)

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)

bla_{TEM}

Among the pools tested by qPCR screening, 82.8% (72/87) were positive for bla_{TEM}. 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 bla_{TEM} in the 72 pools testing positive for bla_{TEM}, 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 bla_{TEM} type/reference genes in ARG-ANNOT site (Figure 1A).

bla_{SHV}

About 18.4% (16/87) of the pools were positive for bla_{SHV}-qPCR. In the homeless group, bla_{SHV} 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 bla_{SHV}, 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 bla_{SHV} in the homeless group (comprising 11 sequence types) and in the control group (comprising 3 sequence types) (Figure 1B).

$bla_{CTX-M-A}$ and $bla_{CTX-M-B}$

None of the DNA pools tested positive for $bla_{CTX-M-A}$ and $bla_{CTX-M-B}$.

Carbapenemase-encoding genes

Only 1 homeless (0.14%) was positive for bla_{OXA-23} by both qPCR and sequencing in nasal swab sampled in 2014 (Figure 1C). None of the DNA pools tested positive for bla_{OXA-58}, bla_{OXA-48}, bla_{OXA-24}, bla_{NDM}, bla_{VIM}.

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 (bla_{SHV} or/and bla_{TEM} or/and bla_{OXA-23}) in the homeless group; the proportion of bla_{SHV} (or bla_{TEM}) 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).

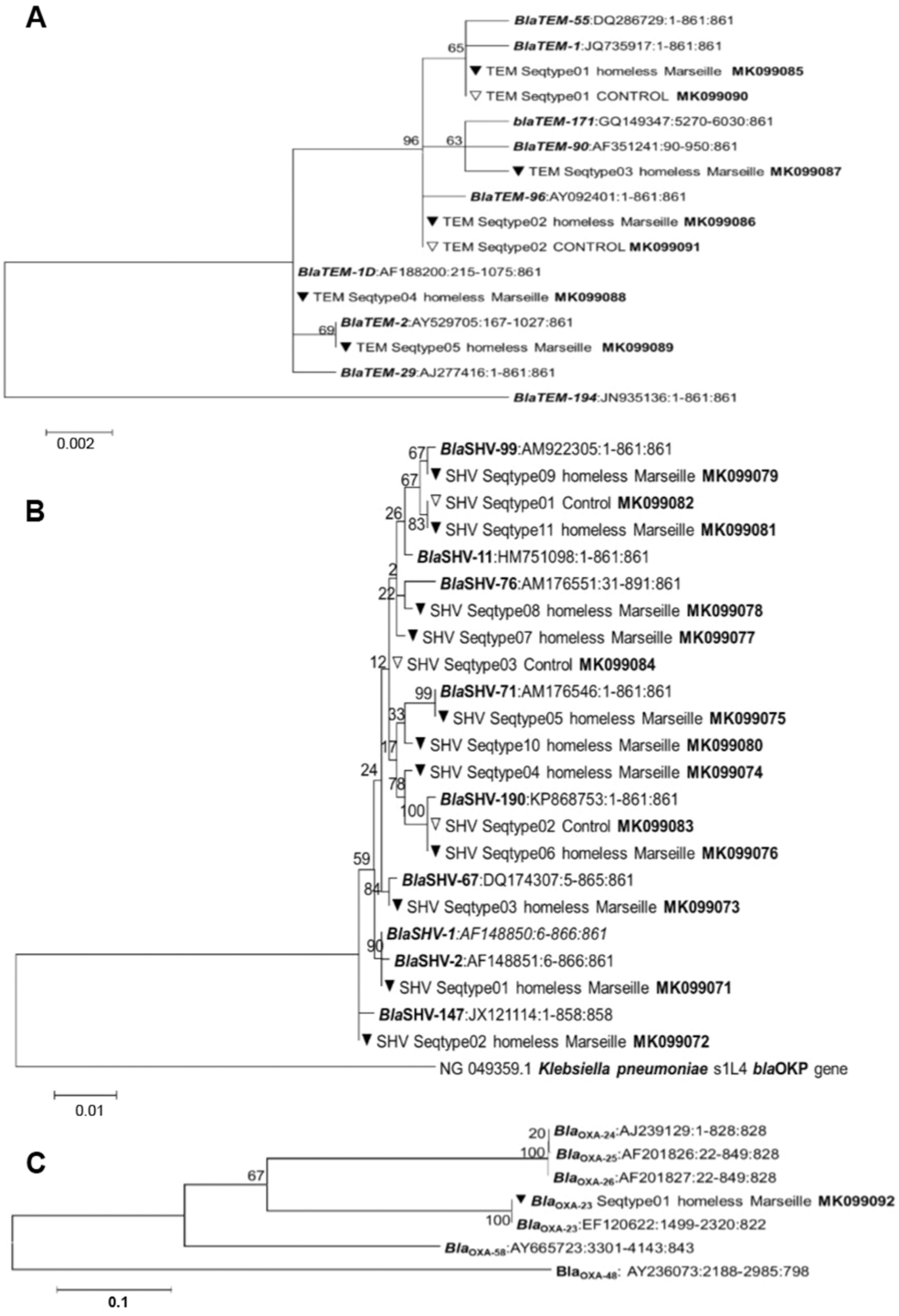

Figure 1 Phylogenetic tree of the diversity of resistance genes detected in nasal/pharyngeal swabs from Marseille homeless people (▾) and comparison group (▽). **(A)** *blaTEM* genotyping; *bla*TEM-194 gene of *K. pneumoniae* (JN935136) was used as outgroup. **(B)** *blaSHV* genotyping; *bla*OKP gene of *K. pneumoniae* (NG049359) was used as outgroup. (C) bla_{OXA-23} genotyping; bla_{OXA-48} gene was used as outgroup. Phylogenetic inferences were conducted in MEGA 7 using the maximum likelihood method based on the Tamura-Nei model.

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[a]				
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
Genre				
Female	13 (1.8)	3 (23.1)	9 (76.9)	-
Male	705 (98.2)	401 (58.0)	290 (42.0)	-
Unknown[b]	6 (-)			
Age at enrolment				
Mean age (SD)	42.8±16 years	N/A	N/A	
Age range (years)	18–84 years	N/A	N/A	
≤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[b]	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[b]	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[b]	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[b]	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*)

Table 2 (Continued).

Characteristics	Total n (%)	At least one resistance gene n (%)	No resistance gene n (%)	Univariate analysis OR (95% CI), *p*-value
Unknown[b]	9 (-)			
Cannabis	126 (17.6)	55 (45.1)	67 (54.9)	0.54 (0.37–0.8), *p*=0.002
Intravenous drug use	3 (0.4)	1 (50)	1 (50)	-
Snorted drug use	22 (3.1)	10 (47.6)	11 (52.4)	-
Drug substitutes	9 (1.3)	3 (33.3)	6 (66.7)	-
Chronic diseases				
Chronic respiratory diseases[c]	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
Unknown[b]	45 (-)			
Seasonal vaccination against influenza[d]	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[e]	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 (0.45–1.13), *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)	

[a]The variable was not included in the analysis, given that no intervention could be done based on this criterion
[b]Unknown: missing data or unidentified samples
[c]Chronic respiratory diseases were defined as suffering from one of the following conditions: asthma, chronic obstructive pulmonary disease, occupational lung diseases and pulmonary hypertension
[d]Proportion of seasonal vaccination against influenza for individuals ≥65 years of age: 24 of 73 participants (32.9%)
[e]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).

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.[20] 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.[21–23] 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.[24]

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 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**		
Genre				
Male	99 (100)	54 (100)	N/A	1.00
Female	0	0		
Mean age (SD)	39.4±17.5	34.4±10.2	N/A	0.06
Age range				
≤30 years of age	47 (47.5)	27 (50.0)	REF	
30-≤50 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				0.08
Europe	22 (22.2)	19 (35.2)	REF	
Africa	74 (72.6)	28 (27.5)	2.28 (1.08–4.85)	0.032
Asia	3 (30.0)	7 (70.0)	0.27 (0.08–1.63)	0.19
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)	N/A	0.117
Addiction				
Tobacco	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[a]	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

[a]Only variables with *p*-values of <0.2 in the univariate analysis

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,[25] 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 β-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 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)
bla$_{TEM}$	396/710 (54.7)
bla$_{SHV}$	27/708 (3.8)
bla$_{CTX-M-A}$	0
bla$_{CTX-M-B}$	0
Carbapenemase encoding-genes	1/708 (0.14)
bla$_{OXA-23}$	1/708 (0.14)
bla$_{OXA-24}$	0
bla$_{OXA-48}$	0
bla$_{OXA-58}$	0
bla$_{KPC}$	0
bla$_{VIM}$	0
bla$_{NDM}$	0
Colistin genes	
mcr-1	0
mcr-2	0
mcr-3	0
mcr-4	0
mcr-5	0

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.

To date, more than 400 members of *bla*$_{TEM}$ and *bla*$_{SHV}$ have been described.[26] It is proven that *bla*$_{TEM-1}$ is one of the major plasmids associated with *H. influenzae* which is a major causative bacterium of community-acquired respiratory tract infections.[27] In a previous work, a high prevalence of *Haemophilus influenzae* (59%) was shown in this population during the period 2015–2017.[28] In this study, similarly high genetic diversity among *bla*$_{TEM}$-encoding strains was observed; each *bla*$_{TEM}$ has one (*bla*$_{TEM-2}$, *bla*$_{TEM-29}$, *bla*$_{TEM-55}$, *bla*$_{TEM-59}$, *bla*$_{TEM-96}$, *bla*$_{TEM-171}$) amino acid substitution when compared to *bla*$_{TEM-1}$.[26] For *bla*$_{SHV-1}$, 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.[29,30] The genetic diversity of *bla*$_{SHV}$-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 *bla*$_{OXA-23}$ 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% *bla*$_{OXA-48}$) when detected by the same molecular method.[18]

Since the first description of mobilized colistin gene *mcr*-1 in a plasmid carried by *E. coli* isolated in China in April 2011,[31] the dissemination of the transposon has been reported in numerous countries across five continents.[32] Few data are available on the prevalence of *mcr*-genes

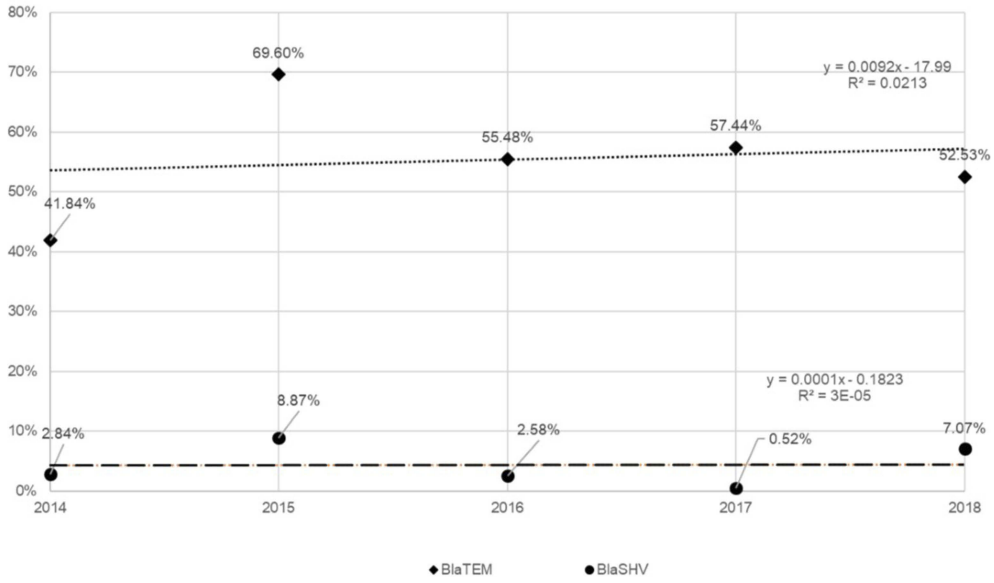

Figure 2 Prevalence of *bla*$_{TEM}$ and *bla*$_{SHV}$ according to the year of study (homeless group).

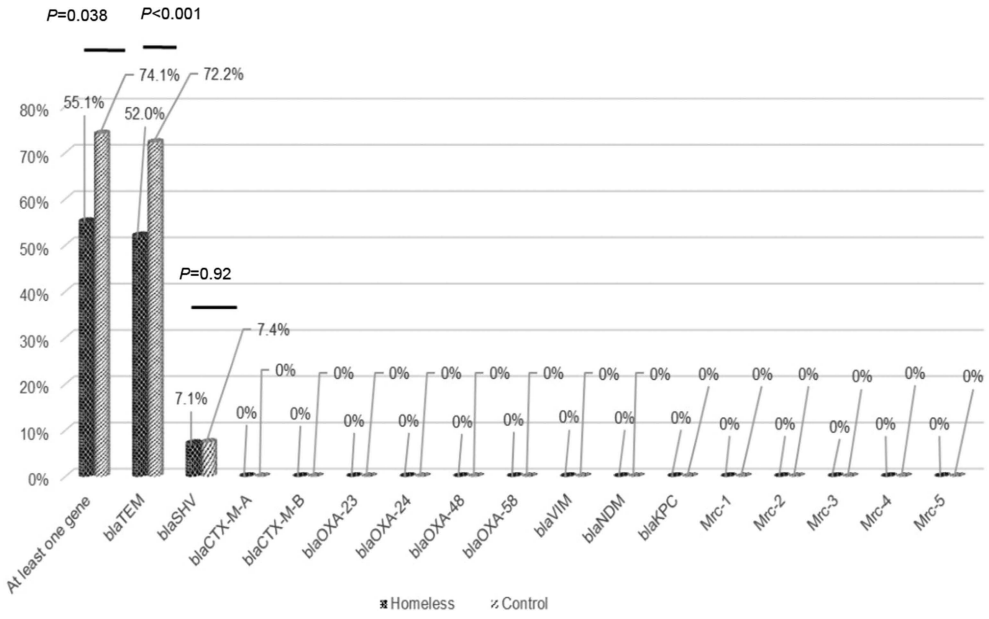

Figure 3 Percentage of individuals harboring antibiotic resistance genes (Comparison between two groups; homeless group: N=98 individuals; controls: N=54 individuals).

Table 6 Multivariate analysis of demographics, chronic medical conditions, clinical findings for resistance gene carriage in homeless group in the period 2014–2018 (N=715 individuals)

Characteristics[a]	Multivariate analysis OR (95% CI), *p*-value
Shelter (A vs B)	1.59 (1.14–2.2), *p*=0.006
Origin	-
Substance use	
Alcohol (frequently vs sometimes or never)	-
Tobacco (yes vs no)	0.55 (0.39–0.78), *p*=0.001
Cannabis (yes vs no)	-
Chronic respiratory diseases	-
Seasonal vaccination against influenza	-
Expectoration	-

[a]Only variables with *p*-values of <0.2 in the univariate analysis

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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