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Short Communication

Methicillin-resistant *Staphylococcus aureus* in the environment of public transport: data from the metropolitan network in Lyon, France

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is involved in community-acquired and nosocomial diseases. The means of MRSA transmission and dissemination in the community remain uncertain. Studies have shown that public transport systems could be a source of MRSA and may serve as a potential source for community-acquired MRSA infections. This study aimed to investigate MRSA contamination on Lyon's metropolitan network (Métro) in France. Hand-touched surfaces were sampled with sterile swabs (Transystem®) during a 1-day transversal study by collecting 50 samples in seven hub stations and two trains for each of the four Métro lines. Then, during a longitudinal study, one sample was collected twice daily for 30 consecutive days in the busiest and most congested hub station. All swabs were incubated in enrichment medium for 24 h and then each suspension was plated onto a chromogenic selective medium for MRSA. After 24 h at 36 °C, all presumptive MRSA colonies were tested using VITEK® MS to confirm identification as *S. aureus* as well as by Alere™ PBP2a Culture Colony Test and *mecA*/*mecC* PCR to check methicillin resistance. Of the 110 swabs tested, 24 presumptive MRSA colonies were isolated, of which 2 were confirmed as *S. aureus* by VITEK® MS. These two isolates were tested negative using the PBP2a Culture Colony Test and PCR. Unlike other foreign cities such as Lisbon, the current data suggest a low level of MRSA contamination of hand-touched surfaces on Lyon's Métro. This should be put in perspective with the low level of MRSA colonisation in the French community.

1. Introduction

Since the beginning of the 1960s, some lineages of methicillin-resistant *Staphylococcus aureus* (MRSA), termed healthcare-associated MRSA (HA-MRSA), have been known to cause nosocomial invasive infections in patients with risk factors. However, during the late 1990s the epidemiology of MRSA infections dramatically changed with the emergence of community-acquired MRSA (CA-MRSA). These latter have become an important worldwide public health issue particularly responsible for skin and soft-tissue infections in healthy populations within the community [1].

Various CA-MRSA clones have been identified, each circulating mostly in one geographical area, e.g. USA300 in the USA, ST80 in North Africa and Europe, ST59 in Asia and ST30 in Oceania [1]. Subsequent worldwide diffusion of these clones has been linked to frequent leisure travel as well as migration flows in a globalisation context. In France, public health institutions are concerned by CA-MRSA local epidemiological changes most likely linked to the high proportion of the population from North Africa. Nevertheless, the specific transmission methods of CA-MRSA within the community remain uncertain. Human colonisation by MRSA is observed especially in the nose and on the hands, therefore its spread in the community is assumed to be mainly due to direct human-to-human interactions and to contact with MRSA-contaminated environments. Interestingly, USA300 clones contain a genetic locus, named arginine catabolic mobile element (ACME), that promotes human colonisation and environmental survival. In this context, public transport, used every day by millions of people, could be a piece of the CA-MRSA epidemiological puzzle. This environment may allow transmission due to the low opportunity for hand hygiene, the high number of hand-touched surfaces and their permanent contact with transports users.

In Lyon, the second biggest city in France, the metropolitan network (Métro) registers ca. 1.5 million trips a day. As no exploration has ever been done on French public transport and because of the demonstrated relationship between CA-MRSA repartition and

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population structure, we describe here an epidemiological investigation of the MRSA contamination of hand-touched surfaces on Métro trains and stations in Lyon, France.

2. Materials and methods

2.1. Sampling

For the 1-day transversal study on the Lyon Métro, surface sampling was performed using Liquid Stuarts Double Swab (Transystem®; Copan Diagnostics, Murrieta, CA). Sampling was conducted while vehicles were in service and at distance from any cleaning period. Sample locations were chosen for having high levels of skin-to-surface contact. A total of 50 surfaces were sampled: 4 handrails and 2 ticket machines in each of the 7 busiest (high attendance and nodal location) Métro stations ($n = 42$ samples) and 2 hand-touched surfaces on 2 different trains for each of the 4 Métro lines ($n = 8$ samples) (Fig. 1A).

For the longitudinal study, sampling was performed twice daily (once in the morning and once in the afternoon, at distance from any cleaning period) for 30 consecutive days in 'Bellecour' station, which is the most crowded station (Fig. 1B).

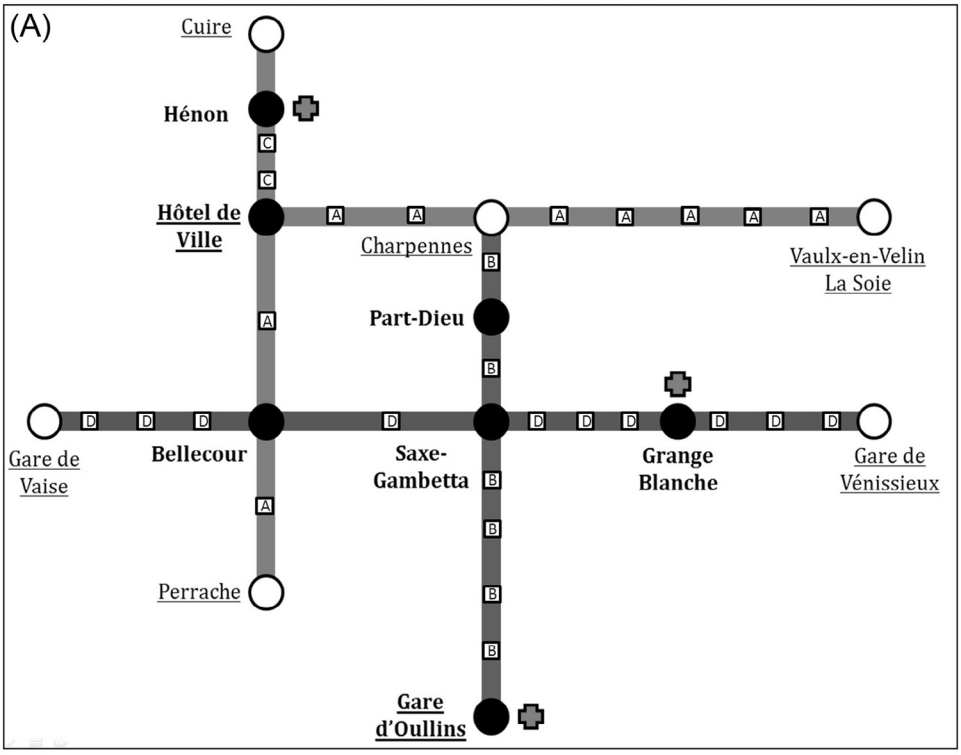
Each swab was dragged along the surface in a manner to maximise surface area contact. After storage at 4 °C for 12–24 h according to the manufacturer's recommendations, all samples were placed in brain–heart infusion (BHI) broth enrichment medium (bioMérieux, Marcy-l'Étoile, France) supplemented with 5% NaCl at 36 °C for 24 h. After enrichment, samples were plated onto chromogenic selective medium for MRSA (chromID™ MRSA; bioMérieux) and were then incubated for 24 h at 36 °C in aerobic conditions.

2.2. Isolation, identification and phenotype characterisation

For all MRSA-suspected colonies (green-blue colonies), matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) identification was performed using VITEK® MS (bioMérieux). For confirmed *S. aureus* colonies, methicillin resistance was explored using Alere™ PBP2a Culture Colony Test (Alere, Waltham, MA) and was confirmed by *mecA*/*mecC* PCR. To investigate associated resistances, antimicrobial susceptibility testing using the disk diffusion method was performed and the results were interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

2.3. Evaluation of the culture process

Before the longitudinal and transversal studies, the ability of the culture protocol to accurately detect *S. aureus* in environmental



(B)

	No. of sites	No. of days	No. of samples
Transversal study	7 stations + 4 lines	1	50
Longitudinal study	1 station ('Bellecour')	30	60

Fig. 1. (A) Scheme of the Lyon Métro. Sampled stations are represented by solid black circles. Squares represent non-sampled stations (A, B, C and D correspond to the line the station belongs to). Crosses represent stations with proximity to a major hospital of Lyon. Empty circles with underlined station names represent the terminus station of Métro lines. (B) Sampling periods. The transversal study screened the main stations of the city during the same day ($n = 50$). The longitudinal study sampled the main hub station in the city with sampling for 1 month twice daily.