



# Isolation of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 30 from house rats (*Rattus tanezumi*) in Hong Kong.

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## ARTICLE INFO

### Keywords:

Antimicrobial resistance  
CA-MRSA  
*Rattus*  
Peridomestic rats  
Public health  
ST 30

## ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is of major public health concern due to its resistance to multiple antibiotics. This resistance has been observed in various settings, including hospitals and communities, and has been detected in both animals and humans. Although peridomestic rat species (*Rattus* spp.) are well described reservoirs of several human pathogens and antimicrobial resistant bacteria, little is known about their role in MRSA epidemiology. In order to investigate whether *Rattus* spp. in Hong Kong are potential carriers of MRSA, 221 rats were caught from various ecological areas and nasopharyngeal samples were cultured on MRSA selective media. Genotypic characteristics of MRSA were confirmed by whole genome sequencing. Two clonal sequence type (ST) 30 MRSA isolates, harbouring *mecA* on staphylococcal chromosome cassette (SCC) *mec* type IVc, were cultured from two house rats (*Rattus tanezumi*) caught in two densely populated urban areas. To the best of the authors' knowledge, this is the first detection of community-associated (CA)-MRSA strain ST30 SCCmec IVc in peridomestic rodents in Hong Kong and globally. Our finding indicates that house rats can be carriers of MRSA strains that are widely distributed in the community.

## 1. Introduction

*Staphylococcus* (*S.*) *aureus* bacteria are both, commensal, and opportunistic pathogens of mammals, persistently occupying specific habitats including the skin, nasal cavities, and various mucosal membranes [1]. Methicillin-resistant *S. aureus* (MRSA) carries a *mec* gene which is associated with resistance to methicillin and to the entire class of beta ( $\beta$ )-lactam antibiotics. That gene is located on mobile genetic elements called staphylococcal chromosome cassettes (SCC). These elements such as SCCmec type II and type III may encompass additional genetic structures that can encode resistance genes to other non- $\beta$ -lactam antimicrobials such as macrolides and aminoglycosides [2–4].

Methicillin-resistant *S. aureus* was first described in 1959 as a nosocomial infection restricted to healthcare settings and referred to as hospital-associated MRSA (HA-MRSA) [5]. Two decades later, community-associated MRSA (CA-MRSA) was reported in a person from the general community with no history of hospitalization [6].

Several animal species carry MRSA, including pet animals, livestock, and wildlife [1,7]. Furthermore, MRSA can be transmitted from animals to humans or vice versa [8,9]. *Rattus* spp. are potential reservoirs of several zoonotic diseases and antimicrobial resistant bacteria [10,11]. Rats may carry MRSA in the oropharynx and rectum [12,13]. Moreover, human-origin MRSA clonal complexes carried by brown rats (*R. norvegicus*) have been identified [12,14].

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<https://doi.org/10.1016/j.onehlt.2024.100861>

Received 5 October 2023; Accepted 17 July 2024

Available online 18 July 2024

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Community-associated methicillin-resistant *S. aureus* (CA-MRSA) is considered to be endemic in the human population in Hong Kong following the first confirmed human case in 2004 [15]. In 2012, there was a significant outbreak of CA-MRSA SCCmec type IV and V infection in a male boarding school and >800 infected cases were reported [16]. A similar outbreak occurred in a neonatal intensive care unit in 2017–2018 and whole-genome sequencing revealed CA-MRSA ST59-SCCmec type V [17]. The increasing number of MRSA infections in the Hong Kong population [18] has raised questions on the potential role of peridomestic animals such as rats in the maintenance and dissemination of MRSA to humans. Hong Kong is one of the most densely populated cities in the world with a severe rat infestation. In 2021, >700 million Hong Kong Dollars (HKD) (~89 million US\$) were allocated to the pest control of the Food and Environmental Hygiene Department [19].

The purpose of this study was to identify and characterize MRSA isolates from peridomestic rats caught from different ecological areas in Hong Kong in order to better understand their potential role in MRSA epidemiology.

## 2. Materials and methods

Rats were trapped alive from 16 different locations in Hong Kong including residential buildings, around street markets, inside livestock farms (pigs and poultry), and horse-riding schools between October 2020 and August 2021. Trapped rats were transported unconscious following the inhalation of 5% isoflurane to the City University Veterinary Diagnostic Laboratory (VDL) for sample collection. The absence of chest movements and responses to noxious stimuli (toe pinch) was recorded and cervical dislocation was performed to confirm death [20]. Following morphometric measurements, the nasal part was cut just in front of the first molar teeth to access the nasopharyngeal cavity, using bone-cutting forceps. Nasopharyngeal samples were collected using sterile swabs, which were individually inoculated into 0.9 ml BHI-broth with 3.5 µg/ml cefoxitin and 65,000 µg/ml NaCl at 37 °C for 24 h. The inoculated broth was streaked on *Brilliance* MRSA 2 agar (Thermo Fisher Scientific, Massachusetts, US) and incubated at 37 °C for another 24 h [21,22]. The presumptive MRSA colonies with denim blue color were harvested for species identification by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry and analyzed by MALDI Biotyper® (Bruker, Massachusetts, US). Cefoxitin disk (30 µg) diffusion test was performed to detect *mecA*-mediated methicillin resistance of the MRSA isolates [23]. *Staphylococcus aureus* ATCC® 25923 was used as quality control strains. During necropsy, liver tissue was also collected and stored at -80 °C for rat species identification as previously described [24].

DNA isolation for complete genome sequencing was performed with the DNA blood and tissue kit (Qiagen, Hombrechtikon, Switzerland) according to the instructions. Paired DNA libraries were prepared using the Nextera DNA Flex Sample Preparation Kit (Illumina, San Diego, CA, USA) and sequencing was performed on an Illumina MiniSeq Sequencer (Illumina, San Diego, CA, USA). The Illumina-read files passed the standard quality checks using the software package FastQC 0.11.7 (Babraham Bioinformatics, Cambridge, UK) and were assembled with Spades 3.14.1 using Shovill 1.1.0 [25,26] with default settings. The assembly was filtered, retaining contigs >500 bp. MLST and Core genome (cg) MLST analyses were performed in Ridom SeqSphere+9.0 (Ridom GmbH, Münster, Germany) with standard settings using draft genomes as input.

SCCmec types were identified using SCCmecFinder 1.2 (<https://cge.food.dtu.dk/services/SCCmecFinder/>) and *spa* type using spaTyper (<http://cge.food.dtu.dk/services/spaTyper/>) [27]. Virulence factors (VFs) were identified by comparing the proteomes with the representative protein data set from the VFDB [28]. A bi-directional-best-hit approach with a 70% identity cut-off was performed using diamond [29]. Similarly, toxins were identified, using toxin sequences downloaded from Uniprot in September 2023 based on a list from Merda et al. [30].

Antimicrobial resistance genes were detected using the resistance gene identifier rgi from the CARD Database [31]. A result with at least 70% identity over at least 70% of the length was considered as a positive hit.

## 3. Results

Four species of rats were identified from 221 individuals including 144 *R. norvegicus* (65%), 67 *R. tanezumi* (30%), 8 *R. andamanensis* (4%), and 2 *Niviventer huang* (1%). Rats were caught from urban areas (47%), livestock farms (43%) and horse-riding schools (10%).

Methicillin-resistant *S. aureus* (MRSA) strains HK255 and HK256 were recovered from nasopharyngeal samples of two immature male Asian house rats (*R. tanezumi*) trapped in urban areas. The first one (19 g) was trapped alive but in a weak condition (exhibiting tremor and lethargy) on February 9, 2021 from the kitchen of a grocery store in Kowloon City District. The second rat (38 g) was caught alive on February 18, 2021 from the back alley of residential buildings in Mong Kok area (Yau Tsim Mong District).

The two MRSA isolates exhibited phenotypic methicillin resistance using cefoxitin discs. Based on the whole genome sequence data both strains were *spa* type t18183 and harboured *mecA* on SCCmec type IVc. Multilocus sequence typing (MLST) revealed that these isolates belong to the sequence type (ST) 30. Core genome MLST analysis revealed that the two isolates were clonal (1 allele difference), whereas the difference to the epidemiologically unrelated isolate MRSA\_TCH60 (GenBank CP002110.1) was 138 alleles (data not shown).

Seventy-one VFs were detected in both strains (Table S1). These included Panton-Valentine leukocidin precursors, making the strains PVL-positive. Further, both strains possessed 7 predicted enterotoxins (Table S2) and resistances against antibiotics such as methicillin, tetracycline, phosphonic acid antibiotics, and fluoroquinolone (Table S3).

## 4. Discussion

This study demonstrates the presence of ST30 MRSA from the nasopharynx of two house rats (*R. tanezumi*) in Hong Kong, highlighting their potential role as reservoirs and disseminators of resistant bacteria. The findings are consistent with previous studies on MRSA in rat species conducted in Canada [12,32], Portugal [33], Germany [34], Austria [35], and China [14]. The two MRSA isolates in this study originated from rats trapped inside and around residential buildings in the densely populated areas of Mong Kok and Kowloon City. These areas represent typical urban areas on the Kowloon side of Hong Kong with multi-story buildings with offices or residential units and including shops, restaurants, bars, and street food on the ground levels. Rats caught in areas with buildings and food gardens are more likely to carry MRSA compared to other areas [36].

Both isolates in this study are CA-MRSA ST30 SCCmec type IV which is the most prevalent type of CA-MRSA in Hong Kong [37]. The isolation of CA-MRSA strains in rats from the kitchen of one grocery shop and the back alley of residential buildings strongly suggests anthropogenic origin. Several studies found that the MRSA strains carried by rats are related to those identified in humans. Rats caught in downtown Vancouver carried the CA-MRSA strain USA300 that circulated in people living in that area [12]. Another study conducted in the south of China revealed that several MRSA strains isolated from rats in Guangzhou City had been previously isolated in a human [14].

In a study from Hong Kong characterizing CA-MRSA, a total of 22 isolates (52.4%) were identified as being ST30 SCCmec type IV. The samples were obtained from various sources including abscesses, pus swabs, wound swabs, tissues, blood cultures, and joint aspirates [38]. In Singapore, CA-MRSA ST30 SCCmec type IV has been identified as the predominant sequence type [39], whereas in Korea it was isolated from a traveler returning from the Philippines [40]. This strain has also been

found to cause severe invasive infections in Japan [41]. In other parts of the world, CA-MRSA ST30 SCCmec type IV has also been described in New Zealand [42], Western Samoa [43], Australia [44], South America [45,46], and Europe [47,48]. In animals, MRSA ST30 has also been isolated from dairy cows with mastitis in South Korea [49] and from a cat admitted to a veterinary clinic in Brazil [50]. However, MRSA ST30 SCCmec IV has never been described in any rat species.

The Asian house rat is a common rodent species in South China and Southeast Asia that lives in close proximity to humans [51], and is therefore readily exposed to human waste, household wastewater, or contaminated materials. Methicillin-resistant *S. aureus* can colonize the rats' nasal epithelium. Previously, a capture-recapture study found that rats may shed MRSA intermittently and become colonized again [13]. The fact that rats could shed MRSA into the environment through their feces [14], together with their gregarious and explorative nature may increase the risk of transmission to people especially within densely populated cities like Hong Kong. In this study, samples were collected only from the nasopharyngeal cavity; therefore it could not be investigated if rats that carried MRSA shed the bacteria through their feces.

## 5. Conclusion

This is the first report of CA-MRSA ST30 SCCmec IV in peridomestic rats in Hong Kong and worldwide. The MLST analysis shows that the molecular characteristics of the isolates are similar to CA-MRSA ST30 described in humans. It can be hypothesized that house rats may serve as reservoirs for CA-MRSA ST30, therefore suggesting potential transmissions between rats and humans in a shared environment which is an important aspect from a One Health perspective.

## Funding

This research was funded by the City University of Hong Kong, start-up grant for new Faculty (project no. 9610449).

## Ethical approval

This study was approved of by the Animal Research Ethics Subcommittee of the City University of Hong Kong (Internal Ref: A- 0380).

## Declaration of competing interest

The authors declare no conflict of interest.

## Data availability

Material has been shared as a supplementary files. Sequencing read data and genome assemblies of the strains HK255 and HK256 have been deposited in BioProject PRJNA1006629 under the accession numbers JAVHUQ000000000 and JAVHUP000000000, respectively.

## Acknowledgements

The authors would like to thank Mr. KAM Ho Yat for the car transport during field work and Mr. WU Wai Hung for his assistance in the field work.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2024.100861>.

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