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Genetic characterization of *Staphylococcus aureus* isolated from Norway rats in Boston, Massachusetts

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Abstract

Background: Despite the importance of domesticated animals in the generation and transmission of antibiotic-resistant *Staphylococcus aureus*, the role of wild animals, specifically rodents, in the ecology of *S. aureus* remains unclear. We recovered and genotyped *S. aureus* isolates from wild Norway rats (*Rattus norvegicus*) in Boston, Massachusetts to examine genetic relationships between common human and animal *S. aureus* isolates in a large US metropolitan area.

Methods: We collected and necropsied 63 rats from June 2016 to June 2017. Nasal, foot pad, fur, and fecal swabs were collected. *Staphylococcus aureus* was isolated using culture-based methods and polymerase chain reaction confirmation. *S. aureus* isolates were *spa* typed, tested for antibiotic susceptibility, and whole genome sequenced. Assembled sequences were uploaded to the Comprehensive Antibiotic Resistance Database to identify antibiotic resistance elements. A phylogenetic tree was constructed using the neighbor-joining method with the maximum composite likelihood distance in MEGA7.

Results: We recovered 164 Gram-positive bacterial isolates from Norway rats. Nineteen isolates from eight individual rats were confirmed as *S. aureus* (prevalence: 12.9% (8/63)). All *S. aureus* isolates were methicillin-susceptible *S. aureus* (MSSA), *pvl*-negative, and resistant to penicillin. Two isolates displayed resistance to erythromycin. Four different *S. aureus spa* types were detected (t933, t10751, t18202, and t189). Thirteen unique antibiotic resistance elements were identified, and all isolates shared genes *mepR*, *mgrA*, *arlR*, and *S. aureus norA*. Phylogenetic analysis if the 19 *S. aureus* isolates revealed they were genetically similar to four clades of *S. aureus* with similar resistance gene profiles isolated from both human- and animal-derived *S. aureus*, as well as formed a distinct phylogenetic cluster composed only of rat isolates.

Conclusions: Wild rodents may serve as a reservoir or vector of antibiotic resistance genes in the urban environment with relevance for human and animal health.

KEYWORDS
drug resistance, *Rattus norvegicus*, *Staphylococcus aureus*, urban health, wild rodent, zoonoses

1 | INTRODUCTION

Staphylococcus aureus is a common Gram-positive bacterium that exists commensally in most mammalian species, residing in the nares, throat, and on the skin of the host (Tong et al., 2015). In humans, *S. aureus* colonizes approximately 30% of the population (Gorwitz, 2008). *S. aureus* can also cause a wide range of infections in humans and is the leading cause of bacterial infections globally in both the healthcare and the community setting (Lee et al., 2018). An individual colonized with *S. aureu* but who does not have an infection, is characterized as an asymptomatic carrier. Carriers are an important reservoir and can be involved in transmission to susceptible individuals through direct personal contact or indirectly through fomites (Senn et al., 2016; Vonberg et al., 2006) or the environment (Katale et al., 2020; Zieliński et al., 2020). *S. aureus* is also a zoonotic pathogen, and direct contact between humans and animals and animal products can transmit the pathogen to humans and vice versa (Algammal et al., 2020; Pirolo et al., 2019; Kadariya et al., 2014). In the environment, *S. aureus* has been recovered in animal manure, water, on surfaces and from the air, highlighting the relevance and diversity of the animal reservoir (Smith et al., 2010).

Studies that characterize *S. aureus* collected from livestock, domestic, and wild animals demonstrate the breadth of animal species that serve as reservoirs of *S. aureus* with relevance to public health, including over 40 species of wildlife (Heaton et al., 2020; Lozano et al., 2016). Furthermore, *S. aureus* collected from humans and animals often share virulence factors, antibiotic susceptibility profiles, and *spa*-strain types, indicating the bidirectional transmission of bacteria and genes (Huijsdens et al., 2006; van Duijkeren et al., 2016). The Panton Valentine leucocidin (*pvl*) pro-toxin subunits lukS and lukF are markers of increased human virulence and *pvl* is commonly detected in the genomes of human-associated MRSA strains and has also been detected in animal-associated strains (Melles et al., 2006). Despite notable research on *S. aureus* among domesticated animals, particularly those raised for food production, there is less research on *S. aureus* carriage among wild animals.

Rodents, particularly rats, are well-adapted to cohabitation with humans in densely populated areas (Santini et al., 2019). Rodent and ectoparasite infestation in urban housing, particularly public housing and homeless shelters, increases risk of pathogen exposure to vulnerable city residents (Byers et al., 2019; Himsworth et al., 2014b; Leibler et al., 2018; McVea et al., 2018). The presence of rodents and rodent exposure among persons living in substandard housing is a well-established health risk, particularly urban youth with asthma (Perry et al., 2003). Rodents may acquire and transmit these pathogens to/from the environment and humans via their scavenging activities in human-generated refuse and urban environments (Himsworth et al., 2014). As such, addressing the role of rodent exposures in public health is a key priority in addressing health disparities.

Recent studies of pathogen carriage in wild urban rodents indicate that these animals harbor bacterial pathogens that can cause human foodborne and hospital-based infections, such as *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Jahan et al., 2021; Lee et al., 2019; Rothenburger et al., 2018). Prior studies have recovered human MRSA strains within wild rodents, indicating that these animals may serve as a vector for human staphylococcal disease (Himsworth et al., 2014). Investigators have recovered both the *mecA* and *mecC* genes, which code for methicillin resistance, in wild rodents (Mrochen et al., 2018; Silva et al., 2021). Due to the rise in antimicrobial resistance, there is a need to better characterize stains of *S. aureus* that circulate in wild rodent populations in urban areas and the risks posed by these pathogens to humans.

In this study, we recovered and analyzed *S. aureus* isolates from wild Norway rats in Boston, MA, a large metropolitan area in the United States, to assess the prevalence of *S. aureus* carriage among rats, to describe the antibiotic susceptibility profile of recovered isolates, and to determine genetic relatedness between *S. aureus* circulating in an urban wild rodent reservoir and profiles of strains commonly detected as human pathogens. The goal of this research was to inform a deeper understanding of the potential role of wild rodents in the ecology of *S. aureus*.

2 | MATERIALS AND METHODS

2.1 | Sample collection and isolation

During the period of July 2016 and June 2017, we collected wild rodents in Boston in collaboration with the City of Boston Inspectional Services Department (ISD). A convenience sampling design was used. Rodents were trapped using Professional Expanded Trigger snap traps with approximately 2 cm pieces of beef jerky for bait at locations with histories of rodent infestations (Back Bay, Chinatown, Long Wharf, Dorchester, Boston Common, North End, Roxbury) in the city of Boston per the existing schedule of ISD. Traps were set and animals collected within 6 h. Specimens were transported under refrigerated conditions for aseptic necropsy and sample collection according to prior protocol (Cummings et al., 2019).

We collected data on rat weight and sex. We acquired nasal, foot pad, fur, and descending colon fecal swabs using Henry Schein 6 dacron-tipped sterile applicators and swabs were immediately streaked for isolation on Remel Columbia Naladixic Acid with 5% sheep's blood (CNA) agar plates (37°C for 24 h) for the isolation of Gram-positive organisms on receipt at the laboratory. One of each distinguishable colony type grown on the CNA agar plates was individually Gram-stained and observed under a light microscope. Colonies of Gram-positive cocci were subsequently plated on Remel™ Blood Agar (37°C for 24 h) and then subcultured onto Mannitol Salt Agar (MSA) and Vogel-Johnson Agar (VJA) (37°C for 24 h) for selective isolation of salt tolerant, coagulase-positive, mannitol fermenting staphylococci (Gómez et al., 2014; Kato et al., 1995). Colonies that were phenotypically consistent with *S. aureus* on MSA and VJA agars were cryopreserved for future analysis. A single colony of each isolated sample was inoculated into 10 ml of Brain Heart Infusion broth and placed into a benchtop incubating shaker for 18 h at 37°C. These colonies were subsequently resuspended in 50% glycerol in a 50 ml conical tube and vortexed with three 2 s bursts at 2000 rpm. One ml aliquots

were stored at −80°C in 2 ml cryogenic storage vials for subsequent characterization.

2.2 | Characterization of isolates

S. aureus isolates were subcultured from glycerol stock on Columbia colistin and nalidixic acid (CNA) plates (Hardy Diagnostics). Isolates grown on CNA plates were confirmed by a series of biochemical tests including the catalase test, coagulase test, and the *S. aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad, Hercules, CA). Isolates positive for the catalase test, coagulase test, and *S. aureus* latex agglutination assay were classified as *S. aureus*.

Genomic DNA from *S. aureus* isolates was extracted using the Wizard Genomic DNA preparation kit (Promega, Madison, WI). Polymerase chain reaction (PCR) was carried out on all *S. aureus* isolates to detect the presence of the methicillin resistance gene *mecA*, which is commonly found on methicillin-resistant *S. aureus* (MRSA) strains that infect humans (Wielders et al., 2002), and the Panton Valentine leukocidin (*pvl*) genes (*lukS, lukF*). PCR was used to amplify *spa* genes using Spa2F (5′-GAACAA-CGTAACGGCTTCATCC-3′) and 1514R (5′-CAGCAGTAGTGCCGTTTGCCT-3′) according to previously published methods (Koreen et al., 2004). PCR was also used to identify seven housekeeping genes (*arcC, aroE, glp, gmk, pta, tpi, yqiL*) associated with multilocus sequence typing (MLST) (Enright et al., 2000). MLST remains incomplete due to time restraints and quality of reads. Isolates with complete reads were assigned sequence types (ST) using the specific MLST database for *S. aureus* (http://saureus.mlst.net).

We used Ridom StaphType software to assign *spa* types (version 2.2.1; Ridom GmbH, Würzburg, Germany). The Based Upon Repeat Pattern (BURP) algorithm was applied to group *spa* types based on their genetic proximity (Mellmann et al., 2007).

2.3 | Antibiotic susceptibility testing

S. aureus isolates were tested for antibiotic susceptibility with the VITEK 2 System (bioMérieux, Durham, NC) using AST-GP76 cards according to manufacturer's instructions, in accordance with the Clinical Laboratory Standards Institute standards (CLSI, 2012). Isolates were tested for susceptibility to penicillin, oxacillin, tetracycline, erythromycin, ciprofloxacin, moxifloxacin, minocycline, clindamycin, trimethoprim-sulfamethoxazole, quinupristin/dalfopristin, gentamicin, levofloxacin, linezolid, daptomycin, vancomycin, rifampin, minocycline, tigecycline, and nitrofurantoin. Any isolates resistant to three or more classes of antibiotics were considered multi-drug resistant (Magiorakos et al., 2012).

Assembled genomic sequences were uploaded to the Comprehensive Antibiotic Resistance Database (CARD) and run against the Resistance Gene Identifier (RGI) to identify antibiotic resistance elements, including genes and nucleotide polymorphisms. The following parameters were selected: DNA sequence, perfect and strict hits only, exclude nudge, and high quality/coverage.

2.4 | Whole-genome sequencing and assembly

Whole-genome sequencing (WGS) was performed on 20 *S. aureus* isolates. Isolate genomic DNA was prepared using Nextera DNA Flex library prep and WGS was performed using paired-end (2×250 bp) sequencing on a MiSeq instrument (Illumina) to an average sequencing depth of at least 50. No custom primers were used. *Staphylococcus aureus* subsp. *aureus* NCTC 8325 (RefSeq NC_007795.1) was used as the reference genome for mapping trimmed reads (https://www.ncbi.nlm.nih.gov/genome/?term=NC_007795) using CLC Genomics (ver. 12). Once mapped, consensus genomic sequences were inferred using the cut-off thresholds of at least five reads per site. We further focused our analyses on protein-coding regions, using blastn to capture the respective sequences (Camacho et al., 2009). Because there were regions of missing coverage in each isolate, to maximize the number of shared sites used in the phylogenetic analysis, we only included genes that were present across all isolates and reference genomes. This resulted in a subset of 1730 protein-coding genes (out of a total of 2767 genes in NC_007795.1) included in the concatenated multiple sequence alignment. The list of genes and their respective names, and the list of genomic reference strains are available in Table 2, respectively. Isolates with the same R number (i.e., Rat **R51**HC2-3-1 and Rat **R51**HC2-2) were recovered from the same rat.

2.5 | Phylogenetic analysis

To determine whether *S. aureus* rat isolates are all closely related to each other or to isolates from other species, we reconstructed a phylogenetic tree using 29 commonly used reference strains (Supporting Information 1). Using the alignment of 1507253 nucleotide positions shared among 49 sequences, a phylogenetic tree was reconstructed using the neighbor-joining method with the maximum composite likelihood distance as implemented in MEGAX (Kumar et al., 2018). Five hundred bootstrap replicas were used to evaluate the reliability of internal branches . We also reconstructed a maximum likelihood (ML) tree using PhyML 3.0 (Guindon et al., 2010) with the substitution model (GTR) selected using the SMS method with the AIC (Lefort et al., 2017), with branch support evaluated using aLRT (Anisimova & Gascuel, 2006). To reduce computational burden, the ML tree was based on a subset of variable sites only (a total of 46930 positions). Both methods yielded essentially the same topologies; thus, we will only show the NJ tree here.

3 | RESULTS

We collected 63 individual Norway rats in this study ($n = 63$). Average rat weight was 188.5 g, and slightly more than half were female (36/63; Table 2). A total of 164 Gram-positive isolates were obtained (Table 1). Nineteen isolates from eight individual rats were identified as *S. aureus*, yielding a prevalence among Norway rats of 12.9% (8/63), based on positive results to the catalase test, coagulase test, and latex

TABLE 1 Characterization of isolates recovered from wild Norway rats in Boston, MA (n = 164 isolates recovered from 63 rodents)

Gram-positive isolates	N (%)
Staphylococcus aureus	19 (11.6%)
Coagulase negative staphylococci (CoNS)	
Staphylococcus arlettae	1 (1.6)
Staphylococcus auricularis	1 (1.6)
Staphylococcus chromogenes	1 (1.6)
Staphylococcus cohnii ssp urealyticus	34 (20.7)
Staphylococcus equorum	9 (5.5)
Staphylococcus gallinarum	9 (5.5)
Staphylococcus hominis ssp hominis	2 (1.2)
Staphylococcus lentus	1 (1.6)
Staphylococcus sciuri	9 (5.5)
Staphylococcus simulans	1 (1.6)
Staphylococcus warneri	5 (3.0)
Staphylococcus vitulinus	2 (1.2)
Staphylococcus xylosus	10 (16.4)
Other species	
Gardnerella vaginalis	4 (2.4)
Aerococcus viridians	8 (4.9)
Kocuria varians	1 (1.6)
Kocuria rosea	1 (1.6)
Leuconostoc mesenteroides ssp cremoris	1 (1.6)
Enterococcus avium	2 (1.2)
Enterococcus faecalis	18 (11.0)
Enterococcus faecium	1 (1.6)
Enterococcus hirae	2 (1.2)
Unidentified (no growth)	22 (13.4)

agglutination assay (Figure 2). Half of the rats with positive isolates were male and half were female. Rats with a positive isolate were heavier than rats without a positive isolate (225 g vs. 183 g), although this difference was not statistically significant. Four rats had a single positive isolate recovered while four rats had more than one positive isolate recovered (Table 2). The majority of rats (seven of eight) with a positive isolate were recovered in the Back Bay (BB) neighborhood of Boston, while one positive rat was recovered from Downtown (DT) (Figure 1). VITEK 2 identification revealed 22 non-*S. aureus* Gram-positive species, 13 of which were coagulase-negative staphylococci (CoNS) (Table 1). All *S. aureus* isolates were resistant to penicillin, and two displayed resistance to erythromycin (*spa* type t18202). All isolates were *pvl*-negative and *mecA*-negative.

We detected five different *spa* types (t18202, t18292, t933, t10751, and t189). Of the 19 isolates, 79% (15/19) were *spa* type t933, and MLST indicated sequence type (ST) 1094. Other *spa* types detected were t10751 and t189. The BURP algorithm identified no linkages between *spa* types, classifying all strains as singletons.

The CARD database and RGI application identified 13 unique antibiotic resistance elements in the genomes of the 19 *S. aureus* isolates; all isolates shared genes *mepR*, *mgrA*, *arlR*, and *S. aureus norA* (Figure 2). Of these antibiotic resistance genes identified, some genes and their functions were correlated with phenotypic results.

3.1 | Phylogenetic analysis

As depicted in the midpoint rooted tre, the isolates from rats split into three distinct clades, with the bootstrap support of 100% each. The majority of isolates from rats were closely related to each other and appeared to be closest to CP009361 isolate (cc30). This clade was composed of 15 isolates recovered from five individual rats. The second clade consisted of three rat isolates, two of which were highly proximal and were recovered from the same rat (Figure 2), and clustered with the LGA251 strain (FR821779). The third clade included a single isolate from a rat that clustered with reference isolates USA400 and with ST1. The observed clustering pattern roughly corresponded with the distribution of antibiotic resistance genes (Figure 2, Supporting Information 2) across isolates, where isolates that shared the same resistance genes were clustered together.

4 | DISCUSSION

The molecular and bioinformatics analyses performed in this study indicate that Boston city wild rodents carry *S. aureus*. These isolates predominately reflected distinct clusters of *S. aureus* within the rodent reservoir and lack the human virulence gene *pvl*, suggesting animal adaptation. However, some recovered rat isolates were similar to strains of potential concern for human health (USA400/ST1) (de Matos et al., 2016) as well as *S. aureus* strains identified in domesticated animal species (Brody et al., 2008; Ko et al., 2019). Additionally, wild rodents also carried non-*S. aureus*, Gram-positive bacterial species capable of causing human disease, including CoNS and enterococci, further highlighting public health risk.

The most frequently observed *spa* type among the *S. aureus* isolates was t933/ST1094, accounting for 79% (15/19) of the isolates. While t933/ST1094 has not been commonly identified in human or animal studies in the past, nor reported in communities or hospitals, it was previously documented in ewes in Tunisia (Said et al., 2017). In addition, this strain was reported in China, Germany, and the Netherlands, according to the Staph Ridom database (http://spa.ridom.de), although not previously in the United States. Although these strains may be independent, it is possible that these isolates were carried and seeded in a number of geographical regions by human, animal, or other transport. Our findings here indicate that the majority of *S. aureus* isolates recovered from our wild rat population are genetically distinct from strains that typically infect humans.

Among the other *spa* types identified, only t189 is reported in publicly available data. *Spa* type t189 was identified in Ohio and Iowa, recovered in environmental samples and carried by daycare

TABLE 2 Antibiotic susceptibility and associated genetic profile of recovered *S. aureus* isolates (*n* = 19) from wild Norway rats in Boston, MA[a]

Rodent ID and location	Isolate ID	Sex, weight (g)	Source	*spa*	MLST	Antibiotic resistance identified
R7, DT	R7F1	M, 150	Fecal	t10751	nd	PEN
R28, BB	R28N4	F, 225	Nasal	t933	1094	PEN
R44, BB	R44NC4	F, 200	Nasal	t933	1094	PEN
	R44F6-C1		Nasal	t933	1094	PEN
	R44F6-C2		Nasal	t933	nd	PEN
	R44P12		Foot pad	t933	1094	PEN
	R44P4-C1		Foot pad	t933	1094	PEN
	R44P4-C2		Foot pad	t933	1094	PEN
R46, BB	R46FC1-1	M, 100	Hair	t933	nd	PEN
	R267C1-2		Hair	t933	nd	PEN
R47, BB	R47NC1-1	M, 300	Nasal	t933	1094	PEN
	R47NC1-2		Nasal	t933	nd	PEN
	R47NC3-1		Nasal	t933	nd	PEN
	R47NC3-2		Nasal	t933	nd	PEN
	R47P12		Foot pad	t933	nd	PEN
R50, BB	R50HC3-1	F, 100	Hair	t933	nd	PEN
R51, BB	R51HC2-2	F, 400	Hair	t18202 (new)	nd	PEN, ERY
	R51HC2-3-1		Hair	t18202 (new)	nd	PEN, ERY
R52, BB	R52NC3	M, 325	Nasal	t189	nd	PEN

Abbreviations: PEN: penicillin; ERY: erythromycin; BB: Back Bay, DT: Downtown (BB and DT are sampling locations in Boston, MA). "nd" indicates missing data. *spa* type t18202 first identified in this study. Positive control used was mecA and *pvl* positive, spa type t9683, and resistant to PEN, OXA, ERY, CIP and LVX.
[a]All recovered isolates were *mecA* and *pvl* negative.

employees, respectively (Thapaliya et al., 2017; Moritz et al., 2015). These observations suggest that while there may be limited similarity to some, more isolated *spa* types of *S. aureus* circulating in humans, the rodent *S. aureus* is relatively distinct, with clear genotypic and phenotypic differences from *S. aureus* circulating among humans.

Antimicrobial susceptibility testing revealed that all isolates were resistant to penicillin. *Spa* type t18202 was additionally resistant to erythromycin (Supporting Information 1). RGI analysis confirmed that all isolates shared *mepR*, *mgrA*, *arlR*, and *norA*. *MepR*, part of the gene cluster, *mepRAB*, encodes mepR, a substrate-binding mepA regulatory protein. MepA is a MATE family multidrug efflux pump, and its presence in *S. aureus* has been shown to decrease tigecycline susceptibility (McAleese et al., 2005). All the *S. aureus* isolates in this study had *mepR*, but none presented the *mepA* gene, which is commonly expressed by MRSA, perhaps reflecting greater adaptation to antimicrobial susceptibility among MRSA compared to MSSA. MgrA is a global regulator, controlling many genes, and also constitutes a regulatory pathways with ArlRS; together these proteins regulate the expression of large surface proteins, like Ebh, a protein that interferes with fibrinogen and prevents clotting and in turn, clumping of bacteria to host tissue. ArlS, a protein histidine kinase, phosphorylates ArlR, and influences *norA* expression. Together, arlRS activates expression of *mgrA*, which represses *ebh* (Crosby et al., 2016).

Resistance among rodent-recovered *S. aureus* isolates to antibiotics used in hospital settings, such as benzylpenicillin and erythromycin, may suggest that these animals may play a role in transmission or perpetuation of antibiotic resistance genes in the urban environment with relevance to human health. Phylogenic analyses highlighted a small number of recovered isolates clustered alongside reference isolates for USA400/ST1, reflecting potential overlap between rodent and clones associated with epidemic MRSA. The rodent-recovered isolates clustered near USA400/ST1 recovered in this study were methicillin sensitive and *pvl*-negative, indicating a clonal departure from this prior epidemic MRSA strain. While our sample size was too small to draw comprehensive conclusions, the phylogenic similarity may suggest a role for wild rodents in the transmission and/or preservation of pathogenic isolates within the urban environment.

Norway rats in our study carried CoNS, a pathogen of increasing relevance to global health as a driver of hospital-acquired infections (Becker et al., 2014). Further genetic analysis of CoNS identified here is needed to examine shared genes to the 19 *S. aureus* isolates. MRSA and CoNS surveillance among wild animal populations in urban areas is necessary to better understand transmission and risk to susceptible humans and animals (Abdel-Moein & Zaher, 2020; Algammal et al., 2020).
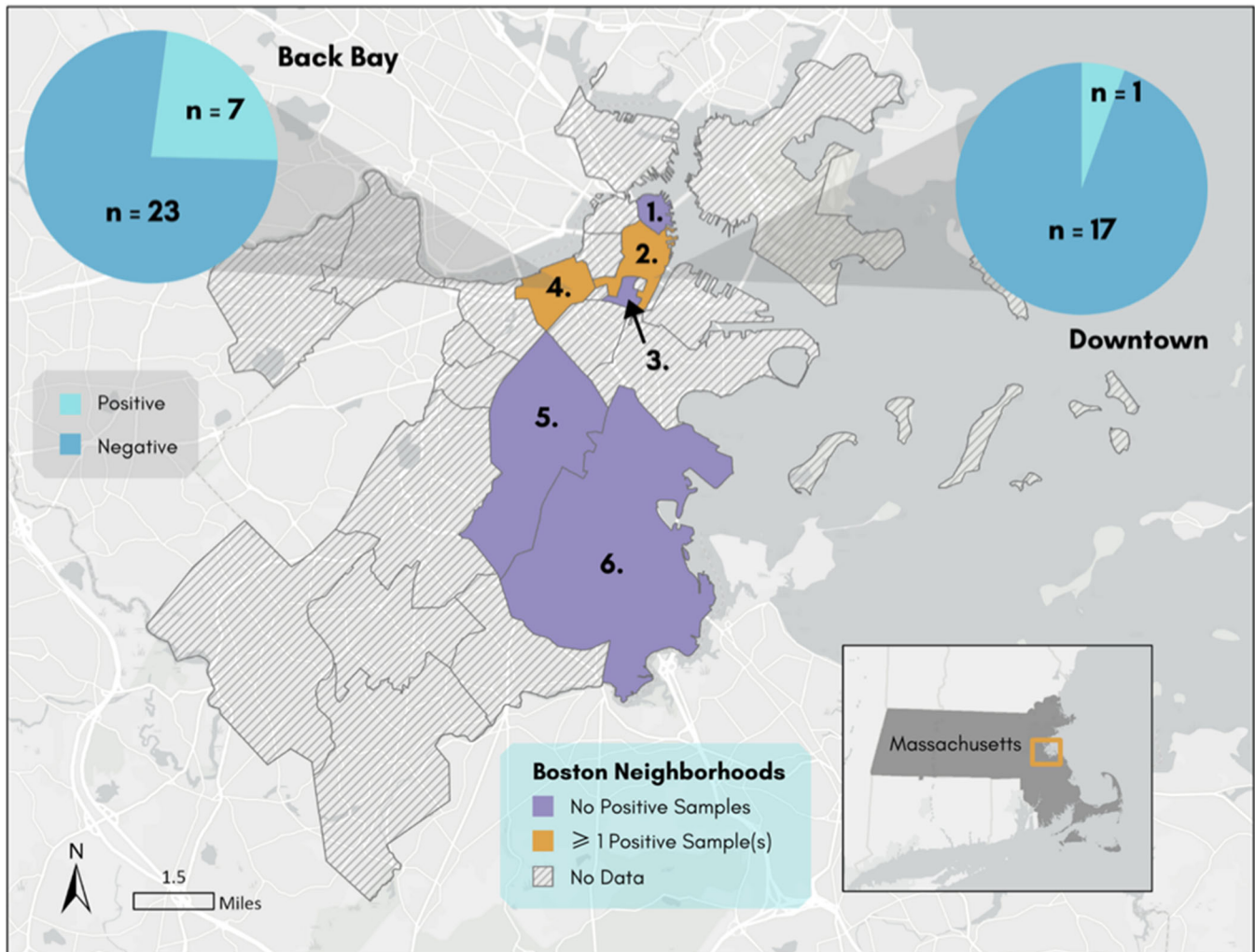
FIGURE 1 Map of neighborhoods in Boston, MA

Generalizability from our study is hampered due to small sample size, given the pilot nature of our research. A larger sample size would provide greater diversity of strain types and may also recover more characteristics of community and hospital-associated *S. aureus* in the wild rodent reservoir. Due to limitations of short-read sequencing, some genomic regions of our isolate draft assemblies—such as those representing repeated sequences (Ben Khedher et al., 2022)—were left unresolved. Our use of latex agglutination assays in support of *S. aureus* identification may be limited by high level of false positives in specimens recovered from some animal species. Our study was likewise limited by the convenience sampling strategy, which relied on active trapping efforts by the City of Boston to acquire rodents. The number of complete whole genomes for *S. aureus* available for analyses provides an additional limitation. On one hand, the number of completed genomes is now quite large and is growing, leading to computational challenges of reconstructing large-scale phylogenetic histories (Aanensen et al., 2016). On the other hand, many published sequences were derived from clinical settings rather than animal surveillance efforts and/or lack comprehensive metadata that describe the origin and source of the sample and what is known about its resistance genes. Such details and background information is essential to provide a comprehensive comparative analysis of the *S. aureus* strains that have been described. Additionally, further research is needed to investigate mechanisms through which rodents may transmit *S. aureus* to humans, or vice versa, in the context of shared environments. Close attention to rodent behavior may elucidate mechanisms of bacterial transmission. How often rodents surface, where and when they search for food, and how they defend themselves from animal/human interaction are questions that may provide clarification towards bacterial dissemination in urban environments.

5 | CONCLUSIONS

Our findings suggest that wild rodents in the City of Boston carry *S. aureus* of relevance to human health as well as distinct strains not commonly reported in the United States that may be specific to the rodent population. Our study contributes to the growing literature on the urban ecology of *S. aureus*, suggesting that wild urban mammals play a role alongside humans and domesticated animals in the evolution
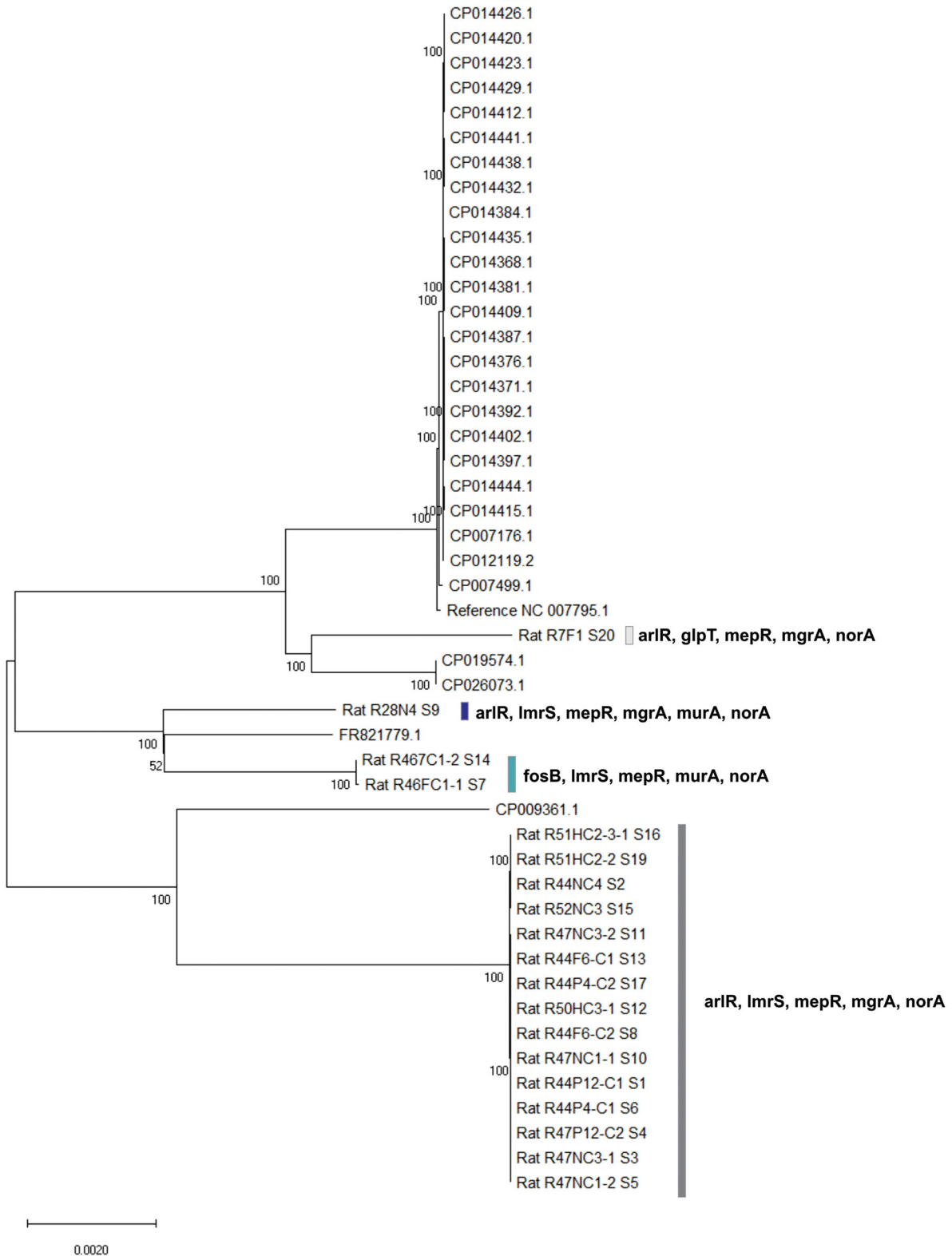
FIGURE 2 Neighbor-joining tree based on the maximum composite likelihood distance that shows clustering of isolates from rats and other reference isolates. Antimicrobial genes recovered from isolates are noted. Isolates from rats are designated with "rat" and a sample number, while other isolates are denoted with the respective GenBank accession numbers, with the exception of reference genome NC_007795.1, used in mapping and assembling. Bootstrap values above 50% are shown as numbers at respective nodes

and ecology of this pathogen within shared urban environments. These pathogens may pose risks for vulnerable populations in urban areas and our study warrants continued study of the animal:human interface in urban centers.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

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