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Genomic Insights into Multidrug-resistant *Mammaliicoccus sciuri* Carrying Novel Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Elements and Plasmids from Farm Ruminants on the East Coast of Peninsular Malaysia

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Abstract

Mammaliicoccus sciuri (formerly known as *Staphylococcus sciuri*) are Gram-positive bacteria that were previously considered as commensals but has since been reported to cause mild to severe infections in both animals and humans. Since very little is known regarding *M. sciuri* isolates from Malaysia, this study aims to characterise the genomes of four *M. sciuri* isolates obtained from the nasal swabs of farm ruminants in the eastern coast states of Kelantan and Terengganu in Peninsular Malaysia. Presumptive *M. sciuri* isolates were validated using MALDI-TOF and antimicrobial susceptibility testing were performed using disc diffusion. The four multidrug-resistant *M. sciuri* isolates were subjected to both short- and long-read genome sequencing using the Illumina and Oxford Nanopore Technologies platforms, respectively. Hybrid assembly led to their complete genome sequences, which revealed diverse genes mediating antimicrobial resistance, heavy metal resistance and virulence, some of which were located on plasmids. All four *M. sciuri* genomes also harboured unique and non-typeable staphylococcal cassette chromosome *mec* (SCC*mec*) elements that encode the *mecA* gene which mediate resistance to β-lactams. Phylogenetic analysis indicated significant diversity within the Malaysian *M. sciuri* isolates and identified close genetic relationships with global isolates from various sources, including humans, suggesting potential cross-species transmission. These findings provide valuable genomic insights into *M. sciuri* from Malaysia and underscore the importance of genomic surveillance in tracking the emergence and dissemination of AMR in this opportunistic pathogen.

Keywords *Mammaliicoccus sciuri* · Farm ruminants · Multidrug-resistant · SCC*mec* · Phylogenetic analysis · Resistance plasmids

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Puteri Nazurah Megat Zakaria and Soo Sum Lean contributed equally to this work.

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Introduction

Domesticated ruminants such as cattle, sheep, and goats, remain one of the major sources of income in Low- and Middle-Income Countries (LMIC), including rural parts of Malaysia. Ruminant livestock farming is prevalently practised by smallholders on the eastern coast of Peninsular Malaysia which comprises the states of Kelantan, Terengganu, and Pahang [1, 2]. Livestock infected with zoonotic pathogens will thus directly impact these small-scale farming communities as well as consumers. The unbridled spread of such infections threatens the income of smallholders, incurs additional veterinary costs and carries the risk of animal to human transmission.

Cross-species transmission of staphylococci in causing zoonosis has been reported worldwide [3, 4]. Staphylococci has been traditionally categorised into coagulase-positive or coagulase-negative staphylococci (CoNS) with *Staphylococcus aureus* being the hallmark of the former category due to its categorisation as a high priority pathogen by the World Health Organization [5]. Five of the CoNS belonging to the *Staphylococcus sciuri* group (*S. sciuri*, *S. fleurettii*, *S. lentus*, *S. stepanovicii* and *S. vitulinus*) had been reclassified under the new genus *Mammaliicoccus* with *M. sciuri* as the type species [6]. Previously considered as commensals and non-pathogenic, *M. sciuri* has since been reported to cause mild to severe infections in both animals and humans [4, 6–9]. Of concern, the isolation of multidrug-resistant (MDR) *M. sciuri* from wildlife and the environment has been increasingly reported in recent years [4, 9, 10].

Horizontal gene transfer is well documented as a major driver of antimicrobial resistance evolution in staphylococci, facilitated by mobile genetic elements such as plasmids, transposons, and staphylococcal cassette chromosome (SCC) elements. The *mecA* gene itself, central to methicillin and β-lactam resistance, was postulated to have originated in CoNS, including species closely related to *Mammaliicoccus*, before being mobilised into *S. aureus* via SCC*mec* [11–13]. The presence of resistance genes in *Mammaliicoccus* spp. thus represents a potential reservoir for staphylococcal pathogens of greater clinical significance. Should these genes be transferred to *S. aureus*, they could further complicate therapy, particularly if linked to multidrug resistance cassettes. Reports of interspecies gene transfer between animal-associated staphylococci and *S. aureus* already highlight the zoonotic and clinical relevance of such reservoirs [14, 15]. Nevertheless, *M. sciuri* has not gained proper attention as most studies primarily focused on healthcare-related staphylococci such as methicillin-resistant *S. aureus* (MRSA), leading to scarcity of data. This disproportion is even more evident in developing countries such as Malaysia. This study thus aims to characterise *M. sciuri* isolated from farmed ruminants on the east coast of Peninsular Malaysia. Genomic characterisation of four of these multidrug-resistant (MDR) isolates was conducted to identify potential links between them and to determine their phylogenetic relationships with publicly available *M. sciuri* genomes. Identification of AMR genes and their associated mobile genetic elements enable us to gain further insights into this lesser known but often MDR zoonotic pathogen from Malaysia.

Methods

Ethical Approval

Ethical Approval for this study was obtained from the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan on 27th October 2022 (Reference: UMK/FPV/ACUE/PG/003/2022).

Bacterial Isolation and Identification

Nasal swabs were collected from healthy goats from Farm A in the state of Terengganu, Malaysia, and Farms B and C in the neighbouring state of Kelantan, as well as healthy cows from Farm D in Kelantan. Swabs were streaked onto Mannitol Salt Agar (MSA) and incubated at 37 °C for 24 h. Resulting colonies exhibiting characteristics consistent with *Mammaliicoccus* spp. (i.e., small, off-white colonies with a yellow halo indicative of mannitol fermentation) were selected for further analysis. Isolates were identified using the MALDI-TOF Biotyper 3.0 (Bruker Daltonics, Germany).

Antimicrobial Susceptibility Test (AST) and Multiple Antibiotic Resistance (MAR) Index

Antimicrobial susceptibility testing of the isolates was conducted using the Kirby–Bauer disc diffusion method following the guidelines set by the Clinical and Laboratory Standard Institute (CLSI) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals (2024; https://clsi.org/shop/standards/vet01/), and the European Committee on Anti microbial Susceptibility Testing (EUCAST, 2025; https://www.eucast.org/ast_of_veterinary_pathogens). To determine methicillin resistance, 1 µg of oxacillin and 30 µg of cefoxitin discs (Oxoid, UK) were utilised alongside *S. aureus* ATCC 25923 as the reference positive control strain. The panel of antibiotics used in this study is as follows: penicillin (10 µg; P10), amoxicillin (30 µg; AMC30), ceftiofur

(30 µg; EFT30), ceftaroline (30 µg; CPT30), azithromycin (15 µg; AZM15), erythromycin (15 µg; E15), clindamycin (2 µg; DA2), amikacin (30 µg; AK30), tobramycin (10 µg; TOB10), gentamicin (10 µg; CN10), quinupristin/dalfopristin (15 µg; QD15), trimethoprim/sulfamethoxazole (25 µg; SXT25), chloramphenicol (30 µg; C30), tetracycline (30 µg; TE30), doxycycline (30 µg; DO30), minocycline (30 µg; MH30), ciprofloxacin (5 µg; CIP5), fusidic acid (10 µg; FD10), norfloxacin (10 µg; NOR10), linezolid (30 µg; LZD30), rifampicin (5 µg; RD5) and enrofloxacin (5 µg; ENR5). The diameter of the inhibition zone was measured in accordance with the CLSI and EUCAST guidelines. Multidrug resistance (MDR) was defined as an isolate that exhibited resistance to three or more classes of antimicrobials [16]. The multiple antibiotic resistance (MAR) index was calculated for each isolate using the formula MAR = a/b, where 'a' represents the number of antibiotics to which the test isolate exhibited resistance, and 'b' denotes the total number of antibiotics tested for susceptibility in the isolate [17].

DNA Extraction and Whole Genome Sequencing

High quality genomic DNA of the four *M. sciuri* isolates were prepared using FavoPrep Genomic DNA Clean-up Kit (Favorgen Biotech. Corp., Taiwan). Genomic DNA concentration was determined using Qubit 2.0 fluorometer (Thermo Fisher Scientitic, USA). Short-read genome sequencing was performed on a MiSeq (Illumina, USA) platform at a commercial sequencing provider (Apical Scientific, Malaysia), yielding 2 × 150 bp paired-end reads. Raw reads obtained were quality assessed and trimmed using fastp (https://github.com/OpenGene/fastp) [18]. For long-read genome sequencing, ca. 500 ng genomic DNA of each isolate was used to construct a DNA library using a Rapid Sequencing Kit (SQK-RAD004) as described by the manufacturer (Oxford Nanopore Technologies (ONT), UK). Sequencing was performed on MK1B (MIN-101B) MinION platform with a FLO-MIN 106 R9.4 (SpotON) flow cell according to the manufacturers' instructions (ONT, UK).

Genome Sequence Analysis

Hybrid assemblies from the short- and long-read data were performed using Unicycler v0.5.1 (https://github.com/rrwick/Unicycler; [19]), followed by evaluation using Quast v5.3.0 (https://github.com/ablab/quast). The assembled genomes were annotated using PROKKA v1.14.5 (https://github.com/tseemann/prokka) and subjected to in-silico species identification with Kraken v1.1.1 (https://ccb.jhu.edu/software/kraken/). The average nucleotide identity (ANI) of the genomes was determined using FastANI package v1.34 (https://github.com/ParBLiSS/FastANI). Multilocus sequence type (MLST) of the *M. sciuri* genomes were obtained using the mlst toolkit (2022 release; https://github.com/tseemann/mlst). Presumptive SCC*mec* were identified from the assembled genomes using staphopia-sccmec (2021 release; https://github.com/staphopia/staphopia-sccmec) and sccmec biotool v1.2.0 (https://github.com/rpetit3/sccmec) and inspected manually.

AMR genes was identified from the assembled *M. sciuri* genomes using AMRFinderPlus v4.0.15 (https://github.com/ncbi/amr) and ABRicate (2020 release; https://github.com/tseemann/abricate). Virulence factors were identified using ABRicate with the pre-downloaded database from the Virulence Factor Database (VFDB) [20]. (Liu et al., 2022The genomes were investigated for the presence of plasmids using PlasmidFinder v2.1.6 (https://github.com/genomicepidemiology/plasmidfinder). Linear plasmid comparison maps were generated using EasyFig v2.2.2_OSX (https://mjsull.github.io/Easyfig/). Graphical representations of ANI statistics (i.e., heatmap) and genotypic AMR (i.e., chord diagram) were generated using in-house R scripts utilizing ggplot2 (https://ggplot2.tidyverse.org/) and circlize (https://github.com/jokergoo/circlize) packages, respectively.

Determination of Concordance Rate between Antimicrobial Resistance Phenotypes and Genotypes

The phenotypic resistance of the *M. sciuri* isolates was compared with the prediction of antimicrobial resistance determined from the identification of resistance genes from their assembled genomes. The concordance rate was calculated as the proportion of concordant isolates from the total number of isolates, with concordant isolates defined as those having phenotypic resistance and the corresponding genotypic resistance determinants in their genomes [21, 22].

Phylogenetic Analysis

A total of 299 *M. sciuri* genomes were downloaded from the National Center for Biotechnology Information (NCBI) public genome database (last accessed 29th November 2024) and combined with the four genomes in this study to undergo pan- and core-genome analysis. Core genome analysis of these genomes ($n = 303$) was performed using Roary v3.11.2 (https://sanger-pathogens.github.io/Roary/). Alignment from the core genome analysis was used to construct a maximum-likelihood phylogenetic tree. Generalized time-reversible (GTR) and CAT models in FastTree v2.1 were applied in the construction of the phylogenetic tree (http://www.microbesonline.org/fasttree/#Install). Annotation and visualization of the constructed phylogenetic tree were

Table 1 Background, antimicrobial resistance profiles and multiple antibiotic resistance (MAR) index of the four Malaysian *Mammaliicoccus sciuri* isolates

Isolate	Source	Location	Resistance pattern*	No. of antibiotics		MAR index
				Resistant	Classes	
MR4	Goat	Farm A, Terengganu	FOX-OX-P-AMC-CPT-FD-TE	7	4	0.29
MR19	Cow	Farm B, Kelantan	FOX-OX-P-AMC-EFT-FD-Te	7	4	0.29
MR27	Goat	Farm C, Kelantan	FOX-OX-P-AMC-EFT-AZM-E-DA-C-FD-Te	11	7	0.46
MR32	Goat	Farm D, Kelantan	FOX-OX-P-EFT-AZM-E-DA-FD-Te	9	6	0.38

*Abbreviations: P, Penicillin; AMC, Amoxicillin-Clavulanate; FOX, Cefoxitin; OX, Oxacillin; CPT, Ceftaroline; EFT, Ceftiofur; FD, Fusidic Acid; E, Erythromycin; AZM, Azithromycin; C, Chloramphenicol; TE, Tetracycline; DA, Clindamycin

Table 2 Genomic features of the four *M. sciuri* isolates sequenced in this study

Isolate	Accession no.	Genome size (bp)	G+C content (%)	No. of plasmids (*n*)	Plasmid name	Plasmid size (bp)
MR4	JBLMMB000000000	2,890,793	32.4	2	pMR-4a pMR-4b	114,219 45,361
MR19	JBLSCO000000000	2,864,827	32.5	1	pMR-19	81,982
MR27	JBLMMC000000000	2,825,294	32.6	1	pMR-27	28,260
MR32	JBLMMF000000000	2,981,323	32.5	3	pMR-32a pMR-32b pMR-32c	47,915 3,901 2,990

carried out using the interactive Tree of Life (iTOL v6; https://itol.embl.de/) [23].

Genome Accession Numbers

The genome sequence data for this study has been deposited at the National Center for Biotechnology Information (NCBI) Genomes database with the following accession numbers: *M. sciuri* MR4, JBLMMB000000000; *M. sciuri* MR19, JBLSCO000000000; *M. sciuri* MR27, JBLMMC000000000; and *M. sciuri* MR32, JBLMMF000000000.

Results and Discussions

Bacterial identification, Antimicrobial Susceptibility and Multiple Antibiotic Resistance (MAR) Index

Four presumptive *M. sciuri* isolates were obtained from the nasal swabs, with three from the state of Kelantan, and one from Terengganu (Table 1). The species of the four isolates was validated as *M. sciuri* using MALDI-TOF MS on the Ultraflex platform with the Biotyper 3.0 database.

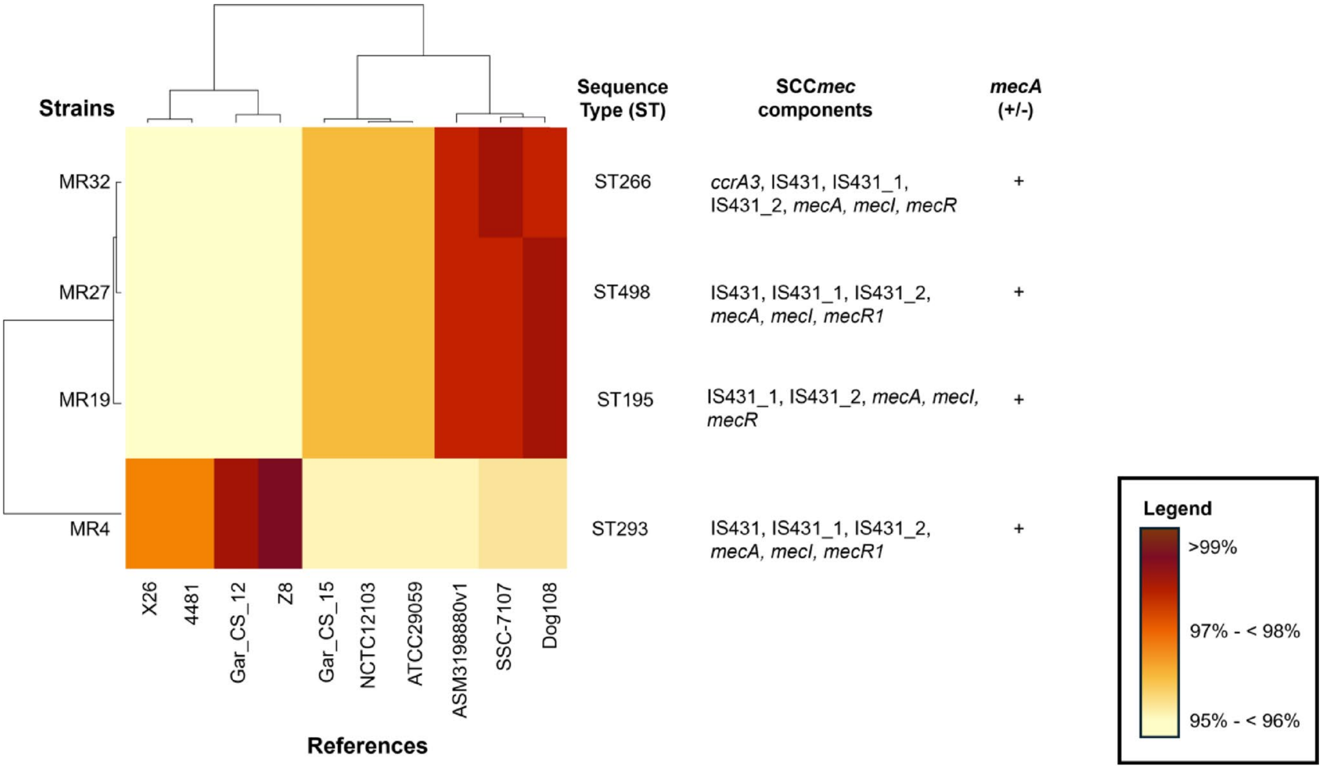
All four *M. sciuri* isolates were then analysed for their resistance to a panel of 24 antibiotics from 15 antimicrobial classes revealing varying levels of multidrug resistance (MDR) with the MAR indices ranging from 0.29 to 0.46 (Table 1; **Supplementary Table S1**). *M. sciuri* MR27 showed the highest level of MDR, with resistance to 11 antibiotics from seven classes. On the other hand, *M. sciuri* MR4 and MR19 had the lowest level of MDR (seven antibiotics from four classes; MAR index of 0.29). The remaining isolate, MR32, displayed an intermediate level of MDR, with resistance to nine antibiotics of six classes (Table 1**).**

Preliminary Genome Analysis

Hybrid genome assembly of the four *M. sciuri* isolates resulted in an average genome size of 2,890,719 bp with average G+C content of ~32.53%. All sequenced isolates consisted of a single circular chromosome with variable number of plasmids (Table 2). Taxonomic identification via Kraken validated the isolates as *M. sciuri* and was supported by ANI and MLST (Fig. 1). MLST analysis showed that the four isolates comprised of four sequence types (STs). All four isolates were singletons with individual STs, namely ST195 (for *M. sciuri* MR19), ST266 (for MR32), and ST293 (for MR4). *M. sciuri* MR27 was a new ST, which was assigned by the PubMLST curators as ST498 (with alleles: *ack-7*, *aroE-16*, *ftsZ-15*, *glpK-20*, *gmk-11*, *pta-9*, and *tpiA-10*) (Fig. 1).

Novel SCC*mec* Elements in the Malaysian *M. sciuri* Genomes

SCC*mec* typing revealed all four *M. sciuri* genomes harboured SCC*mec* elements (Fig. 1) but they were non-typeable. Three of the *M. sciuri* isolates (i.e., MR4, MR19, and MR27) were found to harbour the IS*431-mecA-mecR1-mecI* configuration in the targeted region (Figs. 1 and 2), which resembled the prototypic class A *mec* complex [13]. However, these isolates lacked the *ccr* complex required for SCC*mec* typing and thus resulted in non-typeable cassettes. Nevertheless, the SCC*mec* region of these three isolates were very similar, with two of them (MR19 and MR27) also



Strains	Sequence Type (ST)	SCC*mec* components	*mecA* (+/-)
MR32	ST266	*ccrA3*, IS431, IS431_1, IS431_2, *mecA*, *mecI*, *mecR*	+
MR27	ST498	IS431, IS431_1, IS431_2, *mecA*, *mecI*, *mecR1*	+
MR19	ST195	IS431_1, IS431_2, *mecA*, *mecI*, *mecR*	+
MR4	ST293	IS431, IS431_1, IS431_2, *mecA*, *mecI*, *mecR1*	+

Fig. 1 Heatmap depicting the percentage identity between the sequenced *M. sciuri* isolates in this study (*y*-axis) and reference *M. sciuri* genomes from the database (*x*-axis) that were used in the ANI analysis. Elements detected in the SCC*mec* analysis were as tabulated in the "SCC*mec* components" column. Presence or absence of the methicillin-resistant determinant *mecA*, were marked with '+' or '−', respectively, under the *mecA* column

harbouring both *tetS* and *ant(6)-Ia* resistance genes (Fig. 2). In contrast, *M. sciuri* MR32 was found to harbour the *mec* complex in a IS*431*-*mecA*-*mecR1*-*mecI* class A structure along with a *ccrA3* gene (Figs. 1 and 2). As the current SCC*mec* classification for coagulase-negative Staphylococci (CoNS) requires combinations of either *ccrAB* or presence of *ccrC* for typeability [13], manual inspection of the SCC*mec* cassette from MR32 revealed a novel *ccrB* homolog (Fig. 2). This *ccrB* homolog from MR32 shared 91.3% and 97.8% sequence identity to the *ccrB3* gene from SCC*mec* type III(3A) [13] and the *ccrB* gene from *S. haemolyticus* (accession number: WP_311548572.1), respectively. Comparisons with existing SCC*mec* types showed only weak similarities with SCC*mec* types IX and X (Fig. 2). Therefore, *M. sciuri* MR32 likely harbours a novel SCC*mec* type possibly unique to *M. sciuri* although it remained non-typable in the existing SCC*mec* scheme. Interestingly, the SCC*mec* of MR32 also contained the bifunctional aminoglycoside resistance genes *aac(6')-Ie-aph(2")-Ia* (Fig. 2). Hence, similar to previous reports on *M. sciuri* [7, 24, 25], the genomes from this study also displayed unusual SCC*mec* structures which are thus unclassified, for now.

Core Genome Phylogeny Showed Diversity of the Malaysian *M. sciuri* Isolates

A core genome phylogenetic tree was constructed from the four sequenced *M. sciuri* genomes and all available *M. sciuri* genomes that were in the NCBI Genomes dataset ($n = 299$ as of 29th November 2024). The phylogenetic tree indicated that the global *M. sciuri* genome dataset could be divided into two major clusters designated Clusters 1 and 2 (Fig. 3). Cluster 1 could, in turn, be subdivided into two subclusters 1a and 1b, while 1b itself could be further subdivided into 1b(i) and 1b(ii). Cluster 2 could be subdivided into three subclusters 2a, 2b and 2c, with 2b and 2c being able to be further subdivided into 2b(i) and 2b(ii), and 2c(i) and 2c(ii), respectively. Subclusters 2c(i) and 2c(ii) were the largest, followed by 1b(ii), while 2a was represented by a solitary strain (Fig. 3). One of the striking findings of the *M. sciuri* phylogenetic tree is the interweaving of human origin strains amongst those from animals (both farm and companion animals), food and the environment (Fig. 3), inferring likely cross-infection, transmission, and cross-contamination, as had been previously suggested [26–28].

Three of the four Malaysian *M. sciuri* isolates (MR19, MR27, and MR32) were grouped within the large subcluster

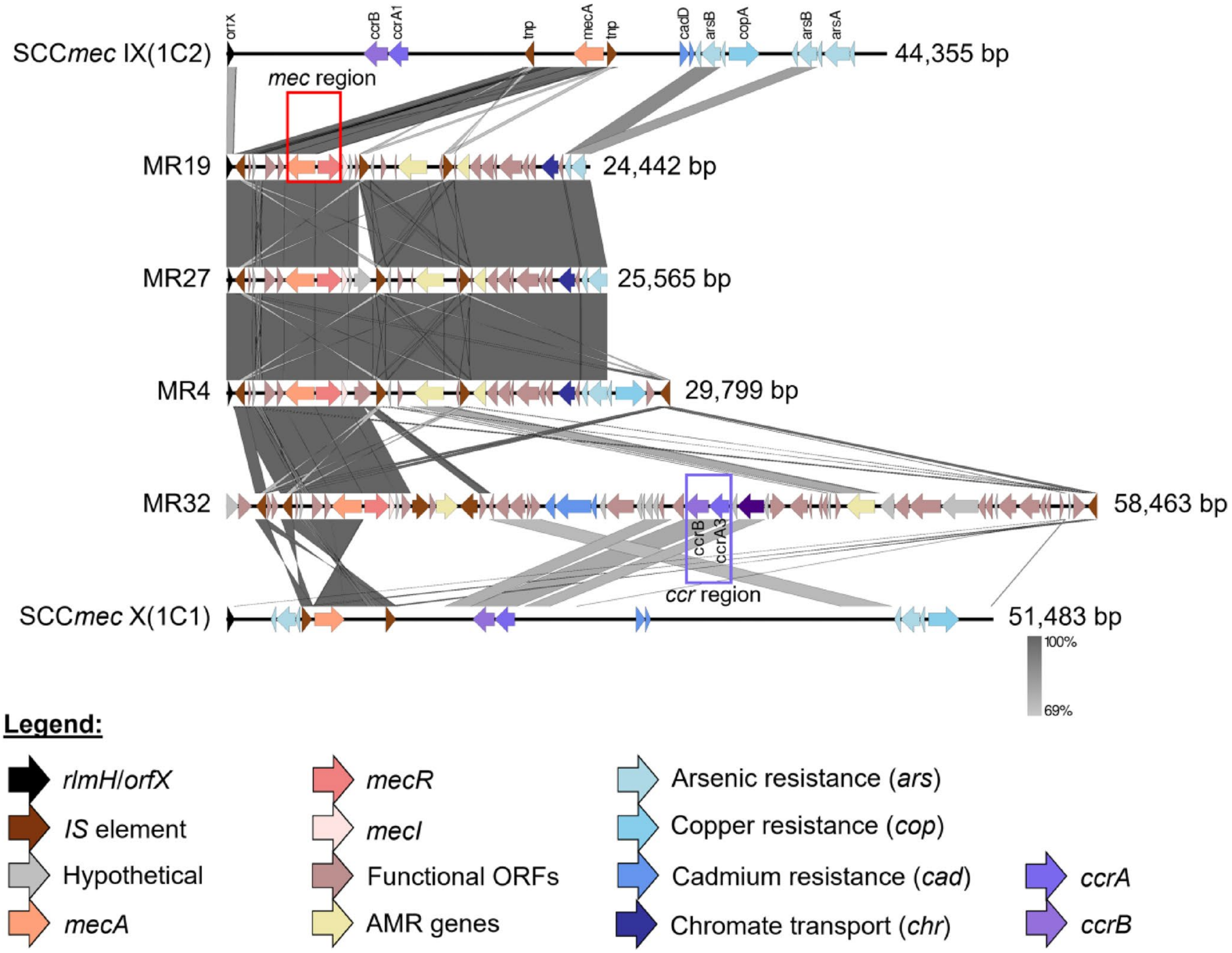


Fig. 2 Comparison of the SCC*mec* cassette region for the four *M. sciuri* isolates with two of the closest SCC*mec* types (i.e., SCC*mec* IX and SCC*mec* X). Strains in this collection carried non-typeable SCC*mec* cassettes, whereby only the *mec* region was found (as labelled) but the *ccr* region was absent. However, MR32 harboured the *ccrA3* and *ccrB* genes which shared partial sequence identities (less than 70%) to the *ccr* region of SCC*mec* X(1C1). Arrows indicate the extent and direction of predicted genes which are coloured according to the legend. Shaded areas in between each linear genetic map indicate regions of >69% nucleotide sequence identities, as shown in the shaded vertical bar at the bottom right of the map

2c(ii). The remaining isolate, MR4, was grouped within subcluster 1b(ii) (Fig. 3). *M. sciuri* MR27 and MR32 were isolated from different goats from the same farm in Kelantan. Although they were grouped within the same large subcluster 2c(ii), MR27 was ST498 whereas MR32 was ST266. A closer look showed that MR32 was grouped together in the same sub-clade with *M. sciuri* Dog108 (isolated from a companion dog in Kenya; [29]) (Fig. 3) which was ST225 and differed from ST266 in four of the seven MLST loci (i.e., *aroE*, *glpK*, *pta1*, and *tpiA*). The nearest neighbour to *M. sciuri* MR27 is strain SSC-7107, which was isolated from the blood of a neonate in Pakistan [30]. SSC-7107 is classified as ST82, which differed to ST498 of MR27 in three loci (i.e., *aroE*, *ftsZ*, and *glpK*). The other Malaysian *M. sciuri* isolate in subcluster 2c(ii), MR19, was grouped together in a sub-clade with *M. sciuri* of other origins and different STs (Fig. 3). This sub-clade consists of *M. sciuri* GNS4y, a human isolate from Assam, India, *M. sciuri* Dog035_2 (from companion dog in Kenya; [29]) and ASM3198880v1 (a metagenome-assembled *M. sciuri* from an unknown source) (Fig. 3). MR19 was ST195 whereas GNS4y was ST333, and Dog035_2 was ST75. *M. sciuri* ASM3198880v1 had an undetermined ST as its assembled genome only matches five of the seven MLST loci (i.e., *aroE*–33; *ftsZ*–12; *gmk*–11; *pta*–9; and *tpiA*–10). ST195 only differed in one locus compared with ST75 (*aroE*–32 for ST195; *aroE*–16 for ST75) but three loci when compared with ST333 (i.e., *aroE*, *glpK*, and *tpiA*).

The remaining Malaysian *M. sciuri* isolate, MR4, which was isolated from a goat in a Terengganu farm and is typed

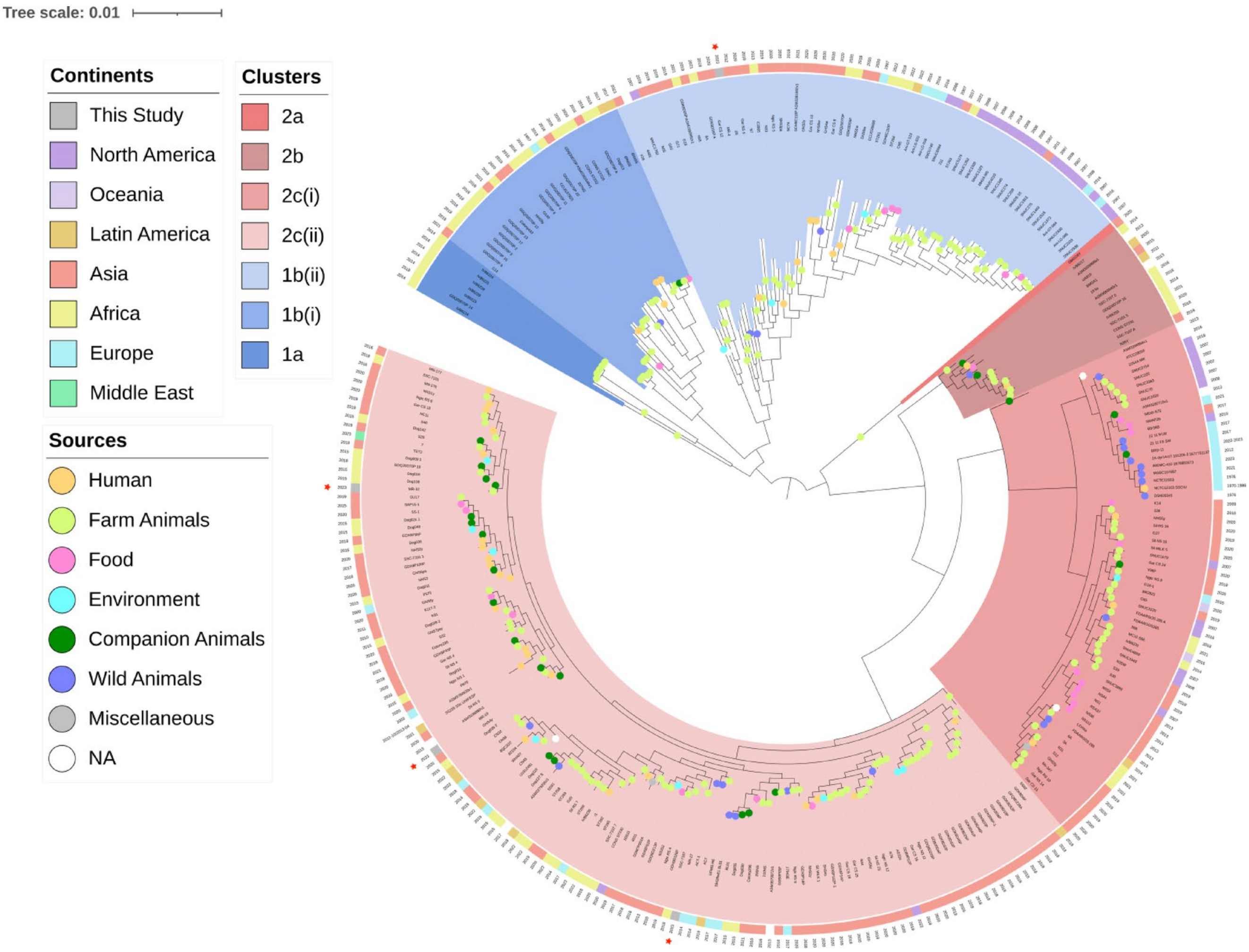


Fig. 3 Midpoint-rooted maximum likelihood core genome phylogenetic tree of global *M. sciuri* isolates ($n=299$) obtained from the NCBI Genomes database along with the four Malaysian *M. sciuri* strains in this study. The red star symbols at the outermost ring indicate the *M. sciuri* isolates from this study. From outer to inner rings are the year the *M. sciuri* isolates were obtained followed by coloured blocks indicating the geographical location of the isolates. This was followed by the name of the isolates themselves which are shaded according to the clusters as observed in the phylogenetic tree. The sources for the *M. sciuri* isolates are indicated in coloured circles at their respective branches of the phylogenetic tree

as ST293, is found in subcluster 1b(ii). MR4 has a close neighbour with *M. sciuri* Z8, an ST45 human isolate from China, and Gar NS 5, an ST307 pig isolate from India (Fig. 3). These STs only differ by two loci, i.e., *pta1* and *tpiA* (for ST293, *pta1*–34, *tpiA*–29; ST45, *pta1*–20, *tpiA*–8; and for ST307, *pta1*–75, *tpiA*–8).

Genotypic and Phenotypic Resistance Profiles of the Malaysian *M. sciuri* Isolates Reveal their Multidrug Resistance (MDR) Traits

Genome sequence analysis of the Malaysian *M. sciuri* isolates led to the identification of multiple antimicrobial resistance genes in each isolate. *M. sciuri* MR27 recorded the highest number of AMR determinants ($n=20$) followed by MR32 ($n=13$), MR19 ($n=8$) and MR4 ($n=7$) as displayed in Fig. 4.

All four *M. sciuri* isolates harboured the *mecA* gene (along with its associated regulatory genes *mecI* and *mecR* in novel SCC*mec* elements as shown in Fig. 2) which conferred resistance to almost all β-lactams, ranging from oxacillin/methicillin to cephalosporins [26, 31–33]. This was reflected in the antibiotic resistance profiles of the four isolates which were resistant to the anti-staphylococcal β-lactams, i.e., penicillin, oxacillin, and cefoxitin (Table 1). Interestingly, three of the four *M. sciuri* isolates were resistant to the third-generation cephalosporin, ceftiofur, with MR4 being susceptible. However, MR4 was resistant to the fifth-generation cephalosporin, ceftaroline, whereas the other three isolates were susceptible (Table 1; **Supplementary Table S1**).

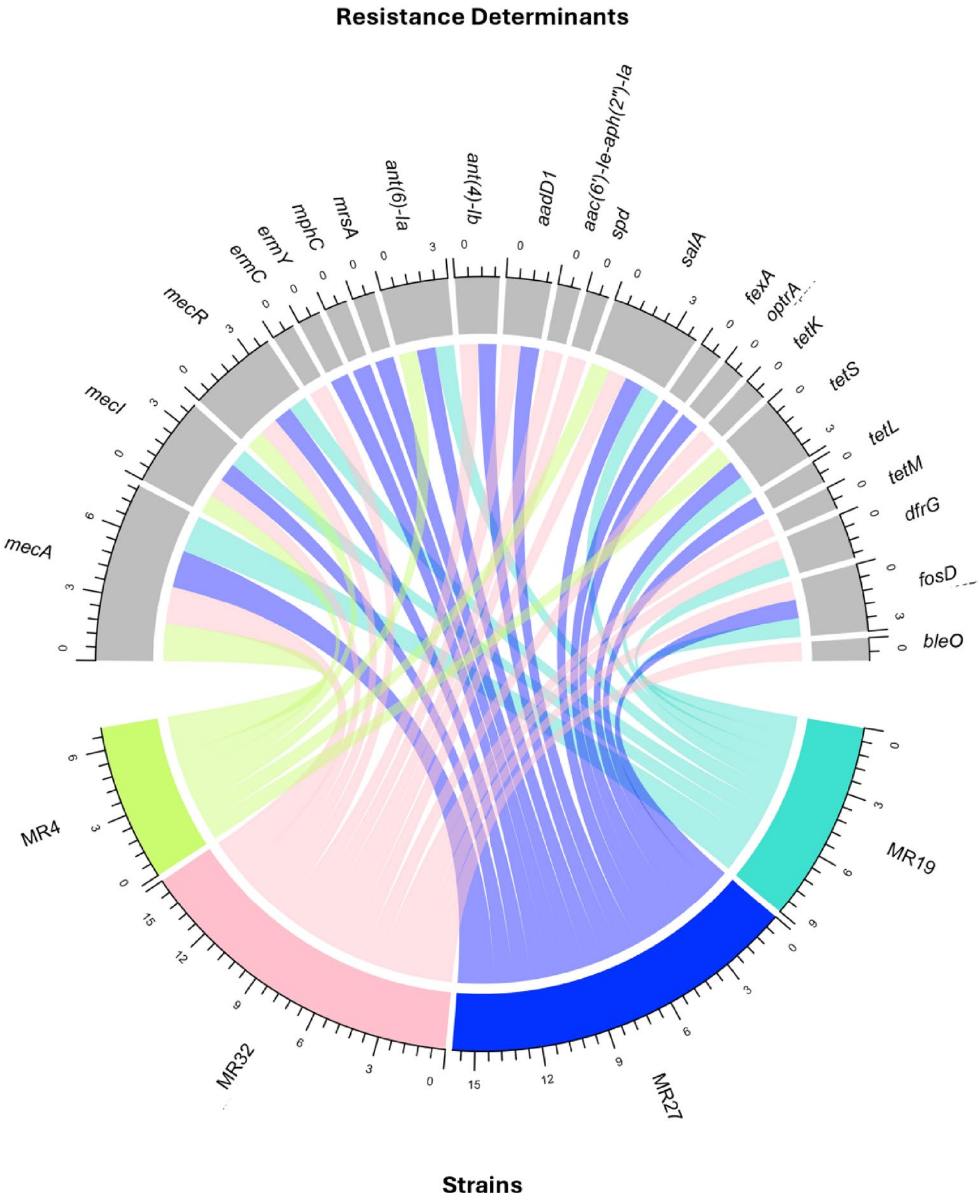


Fig. 4 Chord diagram representation of AMR determinants identified from the four sequenced *M. sciuri* isolates. The isolates (bottom half of the circle) were plotted against the AMR determinants identified (upper half of the circle) using AMRFinderPlus and Abricate. The ribbons linking the strains and AMR determinants were coloured according to each individual isolate; lime green for MR4, pink for MR32, blue for MR27, and turquoise for MR19. Each coloured ribbon which extends out from the isolate represented the presence of that particular AMR gene. The numerical scale showed counts of AMR determinants, based on the number of copies in each isolate

Two of the four *M. sciuri* isolates (i.e., MR27 and MR32) showed resistance towards macrolides and lincosamides, whereas all four isolates were susceptible to the streptogramin, quinupristin/dalfopristin (Table 1). The *erm* genes that encode rRNA methylase are known to mediate macrolide and lincosamide resistance in staphylococci and other Gram-positive bacteria [31, 33, 34]. The *ermC* gene was identified in *M. sciuri* MR32 whereas *ermY* was found in MR27 (Fig. 4). The *salA* gene encodes the SalA ATP-binding cassette (ABC) transporter which functions as an active efflux pump and has been reported to confer *M. sciuri* with resistance to drugs ranging from lincosamide to streptogramin A to pleuromutilin [25, 35, 36]. All four *M. sciuri* isolates harboured *salA* (Fig. 4) but two of these isolates (i.e., MR4 and MR19) were lincosamide susceptible (**Supplementary Table S1**), suggesting that the gene could be inactive or impaired in these isolates. Nevertheless, the true impact of *salA* on lincosamide resistance in *M. sciuri* will require further investigations. On the other hand, macrolide-specific resistance in *M. sciuri* is often mediated by the *mphC* (encoding macrolide phosphotransferase) and *msrA* (encoding an ABC transporter) genes [31, 37, 38]. MR27, which harboured *ermY*, was also found to carry the *mphC*-*msrA* genes (Fig. 4). These observations were in concordance with Matsuoka et al. [38, 39] who reported the co-occurrence of the *mphC*-*msrA*-*ermY* trio of genes that confer macrolide resistance in *M. sciuri*.

The aminoglycoside class of antibiotics (such as gentamicin, amikacin, tobramycin and kanamycin) is widely used, but aminoglycoside resistance remained low and was even reported as a rare trait among CoNS [31, 33, 40]. Genotypic AMR profiles in Fig. 4 showed a variety of genes related to aminoglycoside resistance such as *aac(6')-Ie/aph(2")*, *aadD* and *ant(6)-Ia* in the *M. sciuri* genomes. The *aac(6')-Ie-aph(2")* genes (also known as *aacA-aphD*) are known to confer resistance towards gentamicin, tobramycin, amikacin and kanamycin [41]. The *ant(4')-Ia* gene, also known as *aadD*, was reported to confer resistance towards tobramycin, kanamycin and neomycin [34, 41]. Both the *aac(6')-Ie-aph(2")* and *aadD* genes were identified in *M. sciuri* MR32 (Fig. 4**)**. The co-existence of these genes in MR32, which were found encoded on separate plasmids (which are presented in a later section), could be the reason for its intermediate resistance to gentamicin. The *aadD* gene was also detected in *M. sciuri* MR27 but this isolate was tobramycin and gentamicin susceptible (**Supplementary Table S1**). This observation indicates that the *aadD* gene in MR27 was either inactive or not expressed. Another *aad* gene variant, namely *aadE* (also known as *ant(6)-Ia*), was identified in three of the four *M. sciuri* genomes (where it was absent only in MR32; Fig. 4). However, *aadE* is known to confer resistance to streptomycin [34, 41] and the phenotypic resistance of these *M. sciuri* isolates towards this aminoglycoside was not assessed in this study.

Tetracycline resistance was observed in all four *M. sciuri* isolates whereas intermediate doxycycline resistance was shown in two isolates (MR27 and MR32), and all isolates were susceptible to minocycline (**Supplementary Table S1**). Tetracycline resistance among *M. sciuri* is mainly mediated by efflux pump-coding genes *tetK*, and *tetL* as well as through ribosomal protection protein encoded by *tetM* and *tetS* [34]. The *tetK* and *tetM* genes were detected solely in *M. sciuri* MR32, and this combination of tetracycline resistance genes were previously reported in other *M. sciuri* isolates from farm animals [42]. The *tetS* gene was identified in three isolates, i.e., MR4, MR19, and MR27, while *tetL* was detected solely in MR27 (Fig. 4). *M. sciuri* MR27 thus harboured the *tetL* and *tetS* combination which has yet to be reported elsewhere. Interestingly, the two *M. sciuri* strains that harboured a single *tet* gene (i.e., MR4 and MR19) were susceptible to doxycycline. The other two isolates (MR27 and MR32) that contained combinations of two *tet* genes showed intermediate resistance to doxycycline (**Supplementary Table S1**).

Only one of the four *M. sciuri* isolates, MR27, showed resistance towards chloramphenicol (Table 1), a broad-spectrum nonfluorinated phenicol. Usually, chloramphenicol resistance is mediated by enzymatic inactivation of the drug catalysed by chloramphenicol *O*-acetyltransferases (CATs) but none of the genes that encode CATs such as *catA*, *catB*, or *cat*$_{\mathrm{pC221}}$ [35, 41] were identified in *M. sciuri* MR27. However, the MR27 genome does contain *fexA* which encodes for an active transporter that functions to transport chloramphenicol and florfenicol under induced expression conditions [33, 35, 41]. A high incidence of the *fexA* gene was reported by Schoenfelder et al. (2017) [33] in their study on livestock CoNS where 17 of 19 florfenicol resistant *M. sciuri* isolates (89.5%) harboured the *fexA* gene.

Comparison of Phenotypic and Genotypic Resistance

The concordance rates between the identification of genotypic resistance determinants and phenotypic antimicrobial susceptibility testing varied widely across the antibiotics that were tested in this study (**Supplementary Table S1**). For oxacillin, cefoxitin, and penicillin, the concordance rate was 1.0 with all four *M. sciuri* isolates carrying the *mecA-mecI-mecR* genes also exhibiting phenotypic resistance. This supports the established role of *mecA* as a reliable predictor of resistance to most β-lactam antibiotics in staphylococci [43, 44]. By contrast, concordance rates were considerably lower for some cephalosporins: for ceftiofur, concordance was 0.75, and for ceftaroline, only 0.25. In both cases, the genotypic determinant (i.e., *mecA*) was present in phenotypically susceptible isolates, reflecting false positives. Ceftiofur is a third-generation cephalosporin used in veterinary medicine and the presence of *mecA* in *S. aureus* is usually strongly associated with resistance [45]. Nevertheless, occasional reports of phenotypic ceftiofur susceptibility in *mecA*-positive *S. aureus* suggests factors such as gene expression levels or assay variability may influence concordance between genotype and phenotype for this cephalosporin [45]. Ceftaroline is a fifth-generation cephalosporin which represents an exception to the broad-spectrum resistance to β-lactams conferred by *mecA*. Ceftaroline was specifically designed to overcome PBP2a-mediated resistance by binding to an allosteric site on PBP2a, inducing conformational changes that allow a second ceftaroline molecule to acylate the active site [46]. However, resistance can still emerge through mutations within *mecA* that alter the structure of PBP2a. Mutations in the allosteric domain of the transpeptidase active site have been linked to elevated ceftaroline MICs and clinical resistance in MRSA [47]. Although such research has not been extended to *Mammaliicoccus* spp., these findings in *S. aureus* do explain the divergent concordance rates observed here. While *mecA* reliably predicts resistance to cefoxitin, oxacillin, penicillin, and to a lesser extent, ceftiofur, its predictive value for ceftaroline is weaker since resistance depends on specific *mecA* mutations rather than the mere presence of the gene.

For other antimicrobial classes, including the aminoglycosides amikacin and tobramycin, concordance rates were modest (0.5) even though all isolates were phenotypically susceptible. Such results have been reported for *S. aureus* and the coagulase-negative staphylococci, indicating the likelihood of silent or inactive resistance genes which led to false positives [48–50]. Conversely, some antibiotics such as tetracycline and chloramphenicol showed high agreement (concordance rate of 1.0), indicating the predictive reliability in specific contexts. Nevertheless, the very small sample size in this study ($n = 4$) limits the statistical power and precludes drawing any firm conclusions regarding the predictive reliability of genotypic markers for antimicrobial resistance in *M. sciuri*.

The Virulome of the Malaysian *M. sciuri* Isolates

The four *M. sciuri* isolates contained 12 to 19 virulence factors (VFs) with *M. sciuri* MR32 having the most ($n = 19$) and MR4 having the least ($n = 12$) number of VFs. MR19 and MR27 each encode 17 VFs in their respective genomes (Table 3). Among the various VFs identified, the *ica* gene cluster (*icaABCD* and *icaR*), which encodes adhesins and is implicated in the formation of biofilms in staphylococci [3, 9, 32, 51, 52], was found in all four *M. sciuri* genomes. Most studies on *M. sciuri* also reported the presence of the *ica* gene cluster, in particular *icaA* and *icaC*, which were deemed essential for biofilm production [3, 7, 9, 53]. Besides *ica*, the *sspA* gene was also discovered in all four *M. sciuri* genomes (Table 3). The *sspA* gene encodes for an extracellular proteolytic enzyme and has been reported in all *M. sciuri* isolates studied by Naushad et al. [52] and in *M. sciuri* IMDO-S72 [32]. Two other VFs identified in all four *M. sciuri* genomes are the nucleotide diphosphate kinase-producing gene *ndk*, which functions in phagosome arrest, and the lipoprotein diacylglyceryl transferase-encoding gene *lgt*, which functions as a surface protein anchor (Table 3). Thus, the *ica* gene cluster, *sspA*, *ndk* and *lgt* could be part of the intrinsic VFs that are found in *M. sciuri*.

The *clfB* gene, which encodes clumping factor B, was identified in three of the four *M. sciuri* isolates with the exception of MR4. In contrast, *clfA* (encoding clumping factor A) was absent in all four isolates (Table 3). Naushad et al. (2019) [52] identified *clfB* as one of the most widespread VFs in staphylococci but *clfA* was not detected in the 441 staphylococcal genomes that were analysed. Similarly, *M. sciuri* IMDO-S72 harboured *clfB* but not *clfA* [32]. A comparison of the VFs present in IMDO-S72 and the four Malaysian *M. sciuri* isolates revealed that most were shared, with the exceptions of *alt*, *ebh*, *ebp*, *sdrC*, and *nuc*, which were absent in the Malaysian isolates [32].

Interestingly, several VFs that were identified appear to originate from other bacterial species and this includes *narX* and *sugC* from *Mycobacterium*, *vctC* from *Vibrio*, *lisR* from *Listeria* and allantoin utilisation factor from *Klebsiella* (Table 3). The functionality of these putative VFs and the implications of their presence in *M. sciuri* warrant further investigation.

Table 3 Predicted virulence factors (VFs) of the four Malaysian *M. sciuri* isolates

Categories	Genes	Functions	MR4	MR19	MR27	MR32
Adherence	*clfA*	Clumping factor A	-	-	-	-
	clfB	Clumping factor B	-	✓	✓	✓
	icaA	Intercellular adhesin	✓	✓	✓	✓
	icaB	Intercellular adhesin	✓*	✓*	✓*	✓*
	icaC	Intercellular adhesin	✓	✓	✓	✓
	icaD	Intercellular adhesin	✓*	✓*	✓*	✓*
	icaR	Intercellular adhesin	✓*	✓*	✓*	✓*
Enzyme	*sspA*	Serine V8 protease	✓	✓	✓	✓
Immune evasion	*cap* cluster	Capsule	✓✓	✓✓✓	✓✓	✓✓✓✓
Anaerobic respiration	*narX*	Fused nitrate reductase (*Mycobacterium*)	-	-	✓	-
Cell surface components	*sugC*	Trehalose-recycling ABC transporter (*Mycobacterium*)	-	✓	✓	✓
Iron uptake	*vctC*	Periplasmic binding protein-dependent ABC transport systems (*Vibrio*)	✓	-	✓	✓
Nutritional factor	*cysG2*	Allantoin utilization (*Klebsiella*)	-	✓	✓	✓
	sirB		-	✓	✓	✓
	nirD		-	✓✓	✓	✓✓
Phagosome arresting	*ndk*	Nucleoside diphosphate kinase (*Mycobacterium*)	✓	✓	✓	✓
Regulation	*lisR*	LisR/LisK (*Listeria*)	✓	-	-	-
Surface protein anchoring	*lgt*	Lipoprotein diacylglyceryl transferase (*Listeria*)	✓	✓	✓	✓

Note: A tick mark "✓" denotes a single copy of the virulence gene, with multiple tick marks denotes the number of copies of the same virulence gene. "-" indicates the absence of the virulence gene and an asterisk (*) represents VFs that were discovered from manual search using BLAST

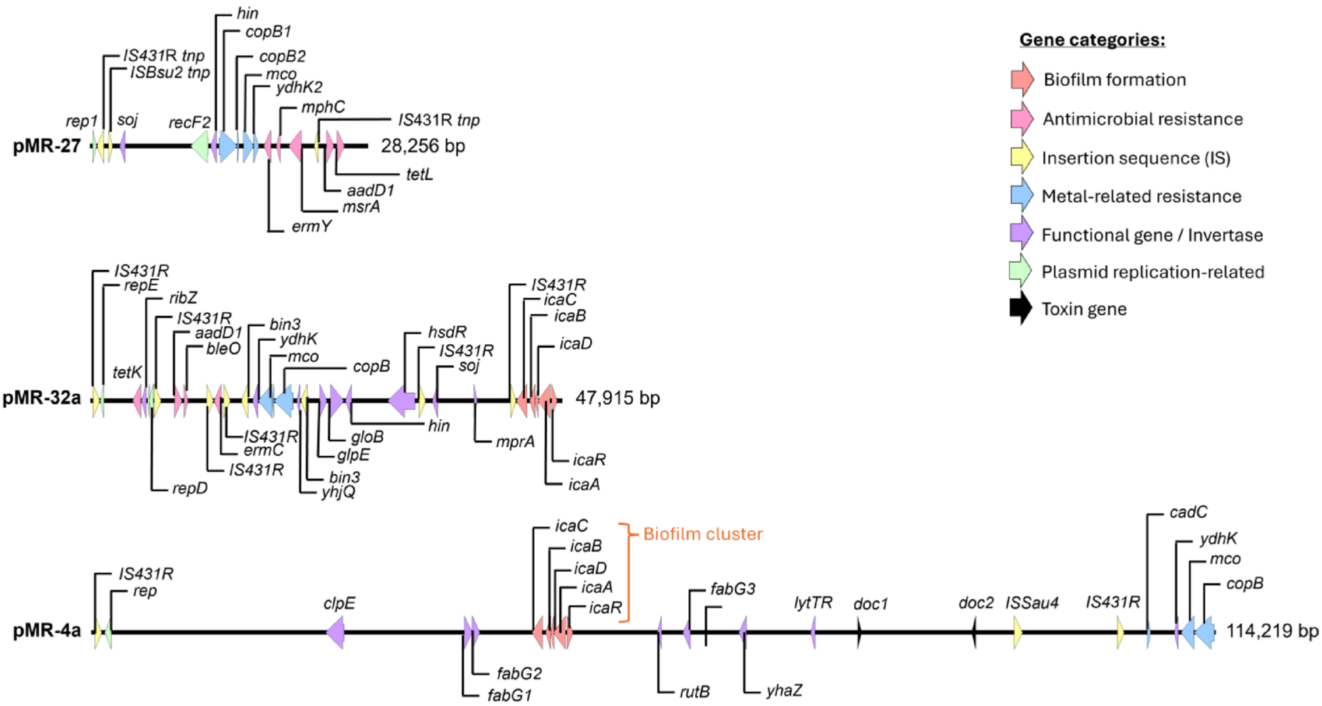


Fig. 5 Linear maps of AMR and/or virulence plasmids identified in the genomes of the *M. sciuri* isolates in this study. Arrows indicate genes with known functions and coloured according to their grouping by categories as indicated

Plasmid Analysis Revealed a Potential Transmissible Arsenal of AMR, metal-resistance, and Virulence Genes in the Malaysian *M. sciuri* Isolates

M. sciuri MR27 harboured a 28,260 bp multidrug-resistant plasmid, pMR27, that encode the *msrA-mphC-ermY* trio of genes mediating macrolide resistance, the *aadA* aminoglycoside, and *tetL* tetracycline resistance genes (Fig. 5). The *msrA-mphC-ermY* trio was first reported on a *S. aureus* plasmid pMS97 from a macrolide-resistant clinical *S. aureus* strain MS8968 isolated in 1971 [38, 39]. In the *M. sciuri* plasmid pMR27, this trio of macrolide resistance genes were flanked by IS*431* in a presumptive composite transposon structure, thus hinting at their mobility.

M. sciuri MR32 harboured a 47,915 bp plasmid pMR32a that contained not only AMR (*tetL*, *aadD*, *ermY*, and *bleO* which confers bleomycin resistance) and copper resistance genes (*mco*, *copB1*, and *copB2*), but also virulence genes (the *ica* operon) (Fig. 5). The *tetL* and *aadD* genes are flanked by IS*431R* while the *ermY* gene is flanked by IS*431* as in pMR27, but with the *msrA* and *mphC* genes absent in this putative composite transposon in pMR32a (Fig. 5). Less than 3 kb downstream of *ermY*, the copper resistance determinants *mco*, *copB1* and *copB2* were found in the same genetic arrangement described in pMR27. Our recent study on clinical MRSA isolates from the same region in Malaysia [54] also reported the prevalence of these genes on plasmids, highlighting the predominance of plasmid-encoded copper resistance among the staphylococci in Malaysia. pMR32a also encoded the complete *ica* operon which function to produce intracellular adhesins for bacterial invasion and colonisation [3, 32]. The *ica* operon was also found on the large 114,219 bp plasmid pMR4a from *M. sciuri* MR4 but this plasmid does not encode any AMR genes (Fig. 5). The *ica* operon has been previously reported to be harboured on large (≥ 40 kb) plasmids in *M. sciuri* [29, 32], similar to our findings here. *M. sciuri* MR4 also harboured a 45,361 bp plasmid pMR4b which does not encode any antimicrobial resistance or virulence genes, but harbour genes that confer arsenic resistance (**Supplementary Fig. S1**). pMR4b also contained a *mob* gene, which indicated the mobilisation potential of the plasmid.

M. sciuri MR32 was also found to harbour two small plasmids, pMR32b (3,901 bp) and pMR32c (2,990 bp) (**Supplementary Fig. S2**). The *spd* gene, which confers spectinomycin resistance, was found on pMR32b, along with the plasmid replication initiation gene designated *repN*, and an open reading frame encoding a hypothetical protein. The smaller pMR32c harbours the *rlmA* gene which encodes an RNA methyltransferase that is responsible for bacterial cell wall formation and virulence [55, 56]. The other gene found in pMR32c encodes for the plasmid replication protein of the Rep_1 family (**Supplementary Fig. S2**).

Conclusions

This study gave us a detailed snapshot of the complete genomes of four MDR *Mammaliicoccus sciuri* isolates from farmed ruminants on the east coast of Peninsular Malaysia. The arsenal of antimicrobial resistance genes identified in these genomes is concerning, particularly given their phenotypic methicillin- and MDR nature. However, the observed discordance between resistance gene carriage and phenotypic susceptibility for certain antibiotics underscores the need for larger studies in *Mammaliicoccus* spp., both to improve the accuracy of genotypic prediction tools and to ensure reliable guidance for antimicrobial therapy and resistance surveillance. The carriage of resistance and virulence genes on novel SCC*mec* elements and plasmids highlights the plasticity of the *M. sciuri* genome and raises the risk of the transmission of these genes into other staphylococcal species of veterinary and clinical significance. This is further underscored by evidence suggesting that CoNS, including *M. sciuri*, serve as reservoirs for various SCC*mec* elements found in the more pathogenic *S. aureus* [13]. Moreover, the lack of clear separation between environmental, animal and human isolates in the global phylogenetic tree of *M. sciuri* is a clear inference of cross-transmission. These findings emphasise the need for a One Health approach and the application of genomic epidemiology to better understand and manage this emerging opportunistic pathogen of concern.

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Data Availability The sequence data for this study has been deposited at the National Center for Biotechnology Information (NCBI) Genomes database with the following accession numbers: *M. sciuri* MR4, JBLMMB000000000; *M. sciuri* MR19, JBLSCO000000000; *M. sciuri* MR27, JBLMMC000000000; and *M. sciuri* MR32, JBLMMF000000000.

Declarations

Competing Interest The authors declare no conflicts of interest.

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