

Whole-Genome Profiling of Coagulase-Negative Staphylococci and Mammaliicoccus from Broiler Chickens: Insights into Mobilome and Resistome Diversity

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Abstract

Staphylococcus species in poultry are emerging opportunistic pathogens and significant reservoirs of virulence and antimicrobial resistance (AMR). In this study, whole-genome sequencing of eight isolates (*Staphylococcus simulans*, *S. shini*, *S. arlettae*, *S. gallinarum*, *Mammaliococcus sciuri*, and *M. lentus*) isolated from infected broiler chickens in India was conducted to analyze their genome comparison and identify plasmids, phages, mobile genetic elements, CRISPR-Cas, antimicrobial resistance and virulence genes. An open pan-genome with 12,200 pan-genes, 243 core genes, and 7,077 strain-specific genes was observed by pan-genome analysis. Plasmid profiling revealed a variety of replicons, including substantial plasmid diversity in *S. gallinarum*, *S. arlettae*, *S. shini*, and *M. lentus* and conserved rep10 and rep7a were found in all isolates. There were 150–193 MGE-associated genes per genome found by mobile genetic element analysis. *Siphoviridae* prophages were only found in *Staphylococcus* species. CRISPR arrays were found in all eight genomes and Type II system (cas1, cas2, cas9) was found in *S. simulans* but *S. shini*, *S. arlettae*, *S. gallinarum*, and *M. sciuri* had Type I systems (cas3). On the other hand, the absence of cas proteins in *M. lentus* suggested a degraded immunological locus. In antimicrobial resistance gene analysis there are 133 AMR genes covering key antibiotic classes were found, and 26 of the genes were conserved in all six species. Additionally, a wide range of virulence factors were found, with *S. simulans* exhibiting the largest profile. These factors included capsular polysaccharides (*cap*), iron-acquisition systems (*sbn*, *isd*), toxins, and secretion machinery (Types II, VI, and VII). All things considered, poultry-derived CoNS and *Mammaliococcus* have large mobilomes, a variety of AMR genes, and fluctuating immunological responses, highlighting their function as hyper-permissive, multidrug-resistant reservoirs with serious zoonotic and veterinary concerns.

Introduction

The genus *Staphylococcus* includes a diverse group of Gram-positive bacteria widely distributed in humans, animals, and environmental niches (Szafraniec et al. 2020). In poultry production systems, staphylococcal infections are associated with skeletal disorders such as osteomyelitis, arthritis, and bacterial chondronecrosis, which contribute to significant economic losses (Szafraniec et al. 2022). Coagulase-negative staphylococci (CoNS) have traditionally been regarded as commensal organisms; however, increasing evidence suggests that they play an important role as opportunistic pathogens and reservoirs of antimicrobial resistance genes (Silva et al. 2023). In addition, several species formerly classified within the *Staphylococcus sciuri* group have been reassigned to the genus *Mammaliococcus* and are now recognized as important carriers of resistance determinants in livestock environments (Ramesh et al. 2024).

The whole gene content of a study group is referred to as a pan-genome. There are numerous uses, such as pathogenicity research, the mobilome, resistome, bacterial lifestyle prediction, and taxonomy. In fact, a reclassification of species is made possible by pan-genome research, which enhances and clarifies the previously established traditional criteria (Caputo et al. 2019). Pan-genome analysis, which makes use of an abundance of genome data, has developed into a representative field for examining the whole repertoire of gene families in the genomes of a particular clade. This has made it possible to develop efficient therapy or engineering strategies for that particular clade. Because pan-genome analysis aids in the development of therapeutic treatments that are effective for the entire clade and effective only for a particular species or strain within a clade based on the biological similarities and differences across the clade, pan-genome studies have demonstrated that pan-genome analysis is one of the effective approaches to better understand a clade of pathogenic bacteria (Kim et al. 2020).

Specific regions of plasmids, known as replicons, encode functions that can initiate and regulate replication. The ability to detect plasmids using raw sequence data or contigs produced by high-throughput sequencing. The proportion of pathogenic or multidrug resistant bacterial clones may be significantly changed by the acquisition of plasmids containing virulence or antimicrobial resistance genes. Therefore, it is crucial to investigate and comprehend the molecular epidemiology of transferable plasmids in addition to the molecular epidemiology of various bacterial clones (Carattoli et al. 2014). CRISPR–Cas systems can be exploited to re-sensitize antibiotic-resistant bacteria by selectively eliminating plasmids that carry antibiotic resistance genes by horizontal gene transfer (HGT) (Tao et al. 2022). Mobile Genetic Elements (MGEs) are discrete regions of DNA that can promote movement within or between bacterial cells. It is divided into types based on their properties and genetic layout and is highly diverse and play a fundamental role in the transmission of genes by capturing accessory genes from the host genome and mobilizing them as a single genetic unit. Consequently, MGEs are major drivers of bacterial evolution, antimicrobial resistance, virulence factors, and metabolic functions (Johansson et al. 2023).

The extensive use of antibiotics in poultry farming has contributed to the emergence of multidrug-resistant bacterial populations. Bacteria can evade antimicrobial activity through multiple mechanisms including enzymatic degradation, target modification, reduced membrane permeability, and active efflux of antibiotics (Schindler et al. 2016). The present study therefore aimed to investigate plasmid, phage, mobile genetic elements, CRISPR-Cas system, virulence screening and antimicrobial resistance profiles of CoNS and *Mammaliococcus* species isolated from broiler chickens.

Materials and methods

Collection and Isolation of coagulase-negative staphylococci (CoNS) and *Mammaliococcus* spp.

A total of 164 swab samples were aseptically collected from the nasal cavity and fibular regions of clinically infected broiler chickens across three geographical regions of India: Tamil Nadu (Vadakkampatti, Udumalaipettai, and Pollachi), Maharashtra (Harsul), and Karnataka (Bengaluru). Samples were transported to the laboratory under cold chain conditions and processed within 1–3 hours of collection. Swabs were inoculated onto mannitol salt agar (MSA) and HiCrome™ *Staphylococcus* selective agar (HiSSA), followed by incubation at 37°C for 24–72 hours. Preliminary identification of

coagulase-negative staphylococci (CoNS) isolates was carried out using Gram staining and standard biochemical tests, including oxidase, catalase, and coagulase assays. Hemolytic activity was also evaluated to determine β - or γ -hemolysis patterns.

DNA extraction

The isolates were grown in LB medium for 24 hours, the genomic DNA was extracted using a Qiagen kit. The quality and DNA concentrations were determined with a NanoDrop 1000 (Thermo Fisher Scientific) and the samples were stored at -20°C until further use.

Polymerase Chain Reaction & Whole Genome Sequencing

The genomic DNA templates were amplified with the *Staphylococcus* spp. specific primer 16S rDNA, and the *S. aureus*-specific primer nuc to exclude *S. aureus*. Further, the 16S rDNA PCR amplicons were subjected to agarose gel electrophoresis with reference standards and visualized via a gel documentation system. The amplicon was then eluted via a QIAquick gel extraction kit (Qiagen 169031925) and sequenced via the Sanger dideoxy method.

For whole genome sequencing, the genomic DNA libraries were prepared for Illumina sequencing via the KAPA DNA HyperPrep Kit according to the manufacturer's instructions. The raw read qualities, such as the sequence and base quality score distributions, average base content per read, GC distribution in the reads and adapters were assessed via FASTQC. On the basis of the report, low-quality sequence reads were trimmed via trimmomatic (version 0.35); by default settings, with a phredcutoff of Q20 (Bolger et al. 2014). The trimmed reads were de novo assembled via SPAdes 3.11.1 and annotated via Prokka 1.12 (Bankevich et al. 2012) (Seemann et al. 2014). The primarily assembled genome was annotated via Prokka to predict genes, CDSs, etc., and the functional annotation was performed via Emapper. NCBI BLAST was performed to predict the closest reference genome of each sample.

Pan-genome analysis

The DOE Systems Biology Knowledgebase (KBase; <https://www.kbase.us/>) and PanExplorer v2 (<https://panexplorer.southgreen.fr/>) were used for pan-genome analysis (Arkin et al. 2018). The Genbank genome files of all eight isolates were created with the Build Genome Set app (v1.7.6), followed by a unified pangenome, isofunctional and homologous gene families were identified by the Compute Pangenome tool (v0.0.7) and KBase Compare Genomes from Pangenome module (v0.0.7), while Pangenome Circle Plot tool (v1.2.0) was used to visualize gene overlaps and correlations.

Orthologous gene identification

Protein sequence data (.fasta format) of six *Staphylococcus* spp. and two *Mammaliicoccus* spp. were used to analyse the Clusters of Orthologous Groups of proteins (COG), protein similarity, phylogenetic relationship, cluster relationship network and expansions in the OrthoVenn3 (<https://orthovenn3.bioinfotoolkits.net/home>) web-based tool (Sun et al., 2023).

Identification of plasmids, phages, mobile genetic elements, and CRISPR-Cas systems

The plasmid identification among the eight species was performed using PlasmidFinder 2.0 (<https://cge.food.dtu.dk/services/PlasmidFinder/>) (Carattoli et al. 2014). The phage genes were analyzed using Phigaro (version) and mobile genetic elements (MGEs) were predicted by a mobile orthologous database (Mobile OG-db). The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) and Cas proteins were analyzed using the CRISPRCasfinder v4.2.20 tool. All these analyses were performed through the Proksee platform (<https://proksee.ca/>) (Najar et al. 2025).

Assessment of antibiotic resistance and Screening of virulence genes

The Antimicrobial resistance genes (AMR) were predicted via the Comprehensive Antibiotic Resistance Database (CARD) (<https://card.mcmaster.ca/>) and virulence genes with Virulence Factor Database (VFDB) (<http://www.mgc.ac.cn/VFs/download.htm>) (<http://www.mgc>)

Results

Isolates and characterization

Among the 164 swab samples collected, 119 CoNS and *Mammaliicoccus* strains were obtained, as shown in Table 1. In the biochemical test, all the strains were Gram-positive with a grape-like structure, the catalase positive, and the coagulase and oxidase tests were negative. None of the isolated strains carried the nuc gene. A total of 13 species were identified from the obtained CoNS and *Mammaliicoccus* strains through 16S rRNA sequencing (Supplementary file).

Table 1
Sample collection from the various regions in India

Region	Area	No. of swab samples	Total no. of strains
Tamilnadu	Vadakkampatti	11	6
	Udumalaipetai	13	5
	Usilampatti	60	33
	Pollachi	10	4
Karnataka	Bangalore	37	36
Mumbai	Maharashtra	34	31
Total no. of samples		164	119

Whole-Genome Sequencing

On the basis of the 16S rRNA sequencing results, eight representative isolates namely, *Mammaliicoccus sciuri*, *M. lentus*, *Staphylococcus arlettae*, *S. shini*, *S. gallinarum*, and *S. simulans* (isolates ALBM_18, KB_08, POL_29, KB_10, M4, M1, M2, and C2) were sequenced for their whole genomes.

Pan-genome analysis

In pan-genome analysis, all eight species exhibited 12200 pan-genes; 243 core genes and 7077 strain-specific genes were identified using PanExplorer v2. In Kbase, the protein-coding genes include 22463 genes with translation, 19147 in homolog families, and 3316 in singleton families. Subsequently, 7419 families, 4103 homolog families, and 3316 singleton families. The Pangenome Circle Plot shows the overall distribution of the eight species, corresponding functional and their genomic overlap shown in Fig. 1a.

Orthologous cluster distribution

In Orthologous gene cluster analysis, a total of 22,297 proteins from the six *Staphylococcus spp.* and two *Mammaliicoccus spp.* were grouped into 3,917 orthologous clusters, including 1,560 single-copy orthologous clusters and overall, 1,358 singleton proteins (6.09%) were identified.

There are 1611 orthologous clusters of conserved core genomes shared among all eight species. These conserved core genomes encode essential housekeeping functions such as DNA replication, transcription, translation, central metabolism, and other fundamental cellular processes. The accessory cluster genes were likely involved in environmental adaptation, metabolism, and strain-specific traits. Uniquely, *S.gallinarum* (M2) displayed the highest genomic attribute count (53), followed by *S.simulans* (C2) (47) and *S.arlettae* (POL-29) (47), as illustrated in the upset plot Fig. 1b.

Genome-specific orthologous clusters

In genome specific orthologous clusters, M4 (*S.shinii*) contained the highest number of orthologous clusters (2,673), suggesting the presence of a variety of different genes and alleles. In contrast, KB08 (*M.lentus*) contained the fewest clusters (2,414). In ALBM-18 (*M.sciuri*), large numbers (338) of singleton proteins were observed, indicating the presence of numerous strain-specific genes. While in C2 (*S.simulans*) and POL-29 (*S.arlettae*), no singleton proteins were detected shown in Table 2.

Table 2
Comparative analysis of Genome-Specific Orthologous Clusters and Singleton Proteins

Species	Protein	Cluster	Singleton
<i>M.sciuri</i> (ALBM-18)	2835	2452	338
<i>S.simulans</i> (C2)	2625	2601	0
<i>M.lentus</i> (KB-08)	2699	2414	250
<i>S.shinii</i> (KB-10)	2967	2694	225
<i>S.gallinarum</i> (M1)	2930	2626	274
<i>S.gallinarum</i> (M2)	2822	2620	184
<i>S.shinii</i> (M4)	2794	2673	87
<i>S.arlettae</i> (POL-29)	2625	2601	0

Phylogenetic relationship

The phylogenetic tree was generated by OrthoVenn3 based on single-copy orthologous gene clustering among the eight genomes. C2 (*S.simulans*) and POL-29 (*S.arlettae*) formed a closely related clade. Likewise, ALBM-18 (*M.sciuri*) and KB08 (*M.lentus*) as well as M1, M2 (*S.gallinarum*) and KB10, M4 (*S.shinii*) and it formed another closely related lineage. These closely related clades represent a high degree of genomic similarity and share a larger proportion of conserved orthologous gene families shown in Fig. 2.

Assessment of plasmid, phage, mobile genetic element and CRISPR-Cas system

Multiple plasmid replicons (rep) were present in all the CoNS and *Mammaliicoccus* spp., indicating considerable variation in plasmid content. Two replicons such as *rep10* and *rep7a* were most frequently detected in all eight species with high nucleotide identities (99–100%).

In M1 (*S.gallinarum*) isolate carried three replicons. In contrast M2 (*S.gallinarum*), POL-29 (*S.arlettae*), KB08 (*M.lentus*) and KB-10 (*S.shinii*) exhibited the highest plasmid diversity which harboring more than three replicons including such as *rep13*, *rep19c*, *rep20*, *rep21*, *repUS43* and *rep22* as shown in Table 3. In phage identification, the *Siphoviridae* family was present in *Staphylococcus* spp. While *Mammaliicoccus* spp. were found to be absent of prophage genes (Supplementary file)

Table 3
Identification of plasmid replicon types using PlasmidFinder

Species	Database	Plasmid	Identity	Query / Template length	Contig	Position in contig	Note
<i>M.sciuri</i> (ALBM-18)	RepL	rep10	100	477 / 477	NODE_76_length_2439_cov_1297.401778	3284..4033	repL(pDLK1)
	Rep_trans	rep7a	99.07	750 / 750	NODE_71_length_5090_cov_1317.102533	1831..2307	repC(pS0385p1)
<i>S.simulans</i> (C2)	RepL	rep10	100	477 / 477	NODE_28_length_2520_cov_687.401302	1585..2061	repL(pDLK1)
	Rep_trans	rep7a	100	945 / 945	NODE_23_length_4507_cov_85.572682	3256..4200	repC(Cassette)
<i>M.lentus</i> (KB-08)	Rep1	rep13	100	924 / 924	NODE_42_length_2063_cov_91.205438	821..1744	repRC(pSK41)
	Rep1	rep21	100	1004 / 1004	NODE_40_length_2516_cov_1911.866339	739..1742	rep(pKH21)
	Rep1	rep22	100	708 / 708	NODE_36_length_5744_cov_1189.010764	2201..2908	repB(pUB110)
	RepL	rep10	100	477 / 477	NODE_39_length_2534_cov_1635.302401	1826..2302	repL(pDLK1)
	Rep_trans	rep7a	96.79	840 / 840	NODE_37_length_5126_cov_3396.415528	3713..4552	rep(pSTE1)
<i>S.shinii</i> (KB-10)	Rep1	rep21	98.21	1005 / 1005	NODE_28_length_2264_cov_928.480110	681..1685	rep(pLNU7)
	Rep1	rep21	96.37	1046 / 1050	NODE_33_length_1220_cov_1349.010499	1..1046	rep(pS0385)
	RepA_N	rep19c	97.23	973 / 972	NODE_12_length_43599_cov_95.846560	36826..37797	rep(pSSP2)
	RepA_N	rep20	96.17	939 / 939	NODE_18_length_16241_cov_115.183185	9294..10232	repA(pSSAP2)
	RepL	rep10	100	477 / 477	NODE_27_length_2534_cov_966.336996	1847..2323	repL(pDLK1)
	Rep_trans	rep7a	100	945 / 945	NODE_23_length_4517_cov_970.572072	3496..4440	repC(Cassette)
<i>S.gallinarum</i> (M1)	Rep1	rep21	96.37	1046 / 1050	NODE_27_length_3040_cov_2511.177523	1..1046	rep(pS0385)
	RepL	rep10	100	477 / 477	NODE_30_length_2534_cov_2367.099715	1847..2323	repL(pDLK1)
	Rep_trans	rep7a	99.89	945 / 945	NODE_21_length_4516_cov_1678.398063	3430..4374	repC(Cassette)
<i>S.gallinarum</i> (M2)	RepL	rep10	100	477 / 477	NODE_14_length_2135_cov_4126.443635	492..968	repL(pDLK1)
	RepL	rep10	100	477 / 477	NODE_14_length_2135_cov_4126.443635	1168..1644	repL(pDLK1)
	Rep_trans	rep7a	99.57	940 / 945	NODE_10_length_5444_cov_2105.974101	620..1559	repC(pKH17)
	Rep_trans	repUS43	100	1206 / 1206	NODE_1_length_1440220_cov_100.707810	686122..687327	CDS12738(DOp1)
<i>S.shinii</i> (M4)	Rep1	rep21	97.99	745 / 1005	NODE_45_length_768_cov_1130.966715	1..745	rep(pLNU7)
	RepL	rep10	100	477 / 477	NODE_19_length_2534_cov_1256.593000	1429..1905	repL(pDLK1)
	Rep_trans	rep7a	100	945 / 945	NODE_16_length_4517_cov_1257.286036	217..1161	repC(Cassette)
<i>S.arlettae</i> (POL-29)	Rep1	rep21	98.73	863 / 1005	NODE_34_length_1543_cov_2047.479536	1..863	rep(pLNU7)

Species	Database	Plasmid	Identity	Query / Template length	Contig	Position in contig	Note
	Rep1	rep22	100	708 / 708	NODE_23_length_5744_cov_1295.107641	1005..1712	repB(pUB110)
	RepL	rep10	100	477 / 477	NODE_28_length_2534_cov_1520.936508	1713..2189	repL(pDLK1)
	Rep_trans	rep7a	97.53	567 / 933	NODE_22_length_8779_cov_1407.856929	7266..7832	rep(pKH7)

In MobileOG-db analysis, the total number of annotated MGE genes across the eight bacterial isolates ranging from 150 to 193 indicated a diverse repertoire of MGE associated genes. Among the isolates, KB10 contained the highest number of MGE-associated genes (193), subsequently M1 (191), M2 (184), ALBM-18 (181), M4 (156), C2 (154), and KB08 and POL29 (150 each). The results show five major categories such as replication/ recombination/ repair, integration/excision, transfer, phage-related functions and stability/transfer/defense. Among these categories, 66–91 genes involved in replication, recombination, and repair followed by 21–63 genes in integration/excision, 13–23 genes in transfer, 12–39 genes in phage-related functions and 8–14 genes in stability/transfer/defense shown in Table 4. Notably, the greatest abundance of integration/excision genes is present in *M.sciuri* (ALBM-18), whereas the highest number of phage-associated (30–39) genes is present in *S.gallinarum* and *S.shinii* (M1, M2 and KB10) shown in Fig. 3. This diverse repertoire of MGE-associated genes in all eight genomes indicates the distribution of antimicrobial resistance and virulence genes through these multiple mechanisms (Johansson et al. 2025).

Table 4
Distribution of MGE- associated genes in CoNS and *Mammalicoccus* spp.

Species	Integration/excision	Replication/ Recombination/Repair	Phage	Stability/Transfer/Defense	Transfer
<i>M.sciuri</i> (ALBM-18)	63	66	18	14	20
<i>S.simulans</i> (C2)	21	91	17	9	16
<i>M.lentus</i> (KB-08)	34	69	12	12	23
<i>S.shinii</i> (KB-10)	41	90	31	11	20
<i>S.gallinarum</i> (M1)	39	86	39	14	13
<i>S.gallinarum</i> (M2)	29	90	30	13	22
<i>S.shinii</i> (M4)	33	84	18	8	13
<i>S.arlettae</i> (POL-29)	25	81	19	9	16

The CRISPR sequences were present in all eight genomes as shown in Table 5a. The presence of CRISPR associates cas protein in *S.simulans* such as cas2 Typel-II-III, cas1 Typell and cas9_Typell and in *S.shinii*, *S.arlettae*, and *S.gallinarum* have cas3_Typel protein. While *M.sciuri* has two cas3_Typel proteins and *M.lentus* was found to be absent of cas protein shown in Table 5b. These genomes with the presence of CRISPR-associated (Cas) proteins, can act as a defense mechanism against mobile genetic elements such as insertion, conjugative plasmids, phages and horizontal gene transfer such as antibiotic resistance genes and virulence genes. (Kandasamy et al. 2024)

Table 5
a Genomic characteristics of CRISPR arrays

Species	CRISPR Start	CRISPR End	CRISPR Length	Consensus Repeat	Repeat Length
<i>M. sciuri</i> (ALBM-18)	13187	13250	63	CTTGTTTGGCCTGTCTTGCAACT	23
	65040	65198	158	CACCTATATCTCATTCCGGAAGGAATGTATTCTAAGAGTTGAAATACTCCC	55
<i>S. simulans</i> (C2)	43951	44381	430	GAATCACTTATACCTAAACTACAGAGTACTAAAC	36
	335679	336107	428	GTTTTAGAATTGTGATCTTTTAGAATGAAGTAAAC	36
	36517	36769	252	ATTGATGAGTACCCCGAGTATTAGGGGACGAGAACT	37
<i>M. lentus</i> (KB-08)	33346	33451	105	AAGGATAACGTTATTGACACGTTTTG	26
	76230	76330	100	GATGACGCTACATCTGATAATGT	23
	118724	118833	109	CGATAAAATAAAAATATCGAACCGCAAA	28
<i>S. shini</i> (KB-10)	31109	31201	92	ATTGCATAAGTCCCCCTAAATTCTAAAGAATT	32
	665433	665531	98	CCATCTGTAACCTGTTGGTTTCCA	24
	413752	413838	86	TAATGATTAGGGGTTCTTAATGCATG	26
	124766	124853	87	ACTAACTCAATAAATTGAGAGTGA	24
<i>S. gallinarum</i> (M1)	620747	620845	98	TGATCAACAAAATACTGAAGCTAA	24
	68755	68853	98	TGGTAATCAACAAGTAACAGATGG	24
<i>S. gallinarum</i> (M2)	577696	577794	98	TTAGCTTTAGTATTTTGTGATCA	24
	44025	44123	98	CCATCTGTTACTTGTGGTTACCA	24
<i>S. shini</i> (M4)	503968	504076	108	TCACTTGAAAATTAACGAAGGAATTTCTTAGTGTTGCT	39
	187810	187911	101	TGGAAATCAACAAGTTACAGATGGTTT	27
	111950	112311	361	GGTTCATCTGGATTTGGCTGCTCGTCTGTTCCATCTCCATTATT	44
<i>S. arlettae</i> (POL-29)	7203	7290	87	CTAATCTTAATAAATTAAGAAGAGC	25

Table 5
b Genomic characteristics of CRISPR-associated (Cas) proteins

Species	Cas-type/subtype	Gene status	System	Type	Begin	End	Strand
<i>M. sciuri</i> (ALBM-18)	cas3_TypeI	forbidden	CAS	CDS	2333	3619	+
	cas3_TypeI	forbidden	CAS	CDS	4503	6542	+
<i>S. simulans</i> (C2)	cas2_TypeI-II-III	accessory	CAS	CDS	45412	45720	-
	cas1_TypeII	accessory	CAS-TypeIIU	CDS	45732	46637	-
	cas9_TypeII	mandatory	CAS-TypeIIU	CDS	47734	49806	-
<i>S. shini</i> (KB-10)	cas3_TypeI	forbidden	CAS	CDS	11277	12800	-
<i>S. gallinarum</i> (M1)	cas3_TypeI	forbidden	CAS	CDS	11297	12796	-

Antimicrobial resistance gene profiles

Genome analysis revealed a total of 133 antimicrobial resistance (AMR) genes across the sequenced isolates. These genes confer resistance to a wide range of antibiotics, including aminoglycosides, fosfomycin, fluoroquinolones, peptide antibiotics, phenicols, diaminopyrimidines, lincosamides, macrolides, nitroimidazoles, aminocoumarins, β -lactams (carbapenems, cephalosporins, cephamycins, monobactams, and penams), rifamycins, mupirocin, sulfonamides, pleuromutilins, tetracyclines, glycolcyclines, glycopeptides, bicyclomycins, nucleosides, phosphonic acids, and streptogramins.

Among these, 26 AMR genes were consistently present across all eight isolates analysed, including *norA*, *AAC(6')-Ie-APH(2'')-Ia*, *AbaF*, *arlR*, *bacA*, *bcrA*, *dfc*, *ermC*, *evgA*, *ImrS*, *ImrB*, *macB*, *murA*, *novA*, *optrA*, *pmrF*, *poxA*, *ranA*, *rlmA(II)*, *rpoB2*, *sul4*, *taeA*, *tet*, *van*, *ykkC*, and *ykkD* (Fig. 4).

Virulence Factor Profiling

Virulence factor analysis revealed the presence of *eno* (enolase) in *M. sciuri*, *M. lentus*, *S. arlettae*, *S. shini*, and *S. simulans*. The *tuf* and *tufA* genes encoding elongation factor were detected in all isolates except *S. gallinarum*. The *bpsC* gene, associated with immune modulation, was present in *M. sciuri*, *S. arlettae*, *S. gallinarum*, and *S. simulans*.

Capsular polysaccharide synthesis genes, including *cap8O*, *cap8B*, *cap8D*, *cap8E*, *cap8G*, *cap8P*, *capN*, *cap8M*, *cap8L*, *cap8K*, *cap8J*, *cap8I*, *cap8H*, *capA*, and *cap5D*, were detected in all the isolates except *M. lentus*. The immune-modulatory genes *wecB* and *galU* were present in all the isolates except *S. gallinarum*.

Iron acquisition and metabolic genes included the *sbnA-sbnI* cluster, *sirA-sirC*, *harA*, *isdB*, *isdF*, *isdI*, *sfaB*, and *sfaC*. Multiple exoenzymes (*aur*, *lip*, *coa*, *spIB-splF*, *hysA*, *geh*, *sspA-sspC*, *gelE*, *sprE*) and adhesion-related genes (*ebp*, *atl*, *aaa*, *clfB*, *sdrD*, *sdrE*, *srtC*, *fss1*, *fss2*, *plr/gapA*, *efaA*, *pilB*) were identified.

The immune modulation and toxin-related genes included *adsA*, *ebh*, *rfaA*, *rfaB*, *cpsA*, *hlgA-C*, *lukE/D/G/H*, *hla*, and multiple superantigen-like genes (*set1-set39*, including *ss10*). The stress survival genes included *clpP*. Secretion systems associated with effector delivery include type II (*gspF*), type VI (*clpV1*, *tagF*) and type VII (*esaG*, *esaE*, *esxB*, *esxD*, *essC*) systems. Biofilm-associated genes (*icaA-D*, *icaR*, *sasC*, *fsrA-C*, *bopD*) and *flgH* were also detected. *Staphylococcus simulans* presented the most extensive virulence repertoire.

Discussion

The present study provides a comprehensive investigation of antimicrobial resistance and virulence factors in coagulase-negative staphylococci (CoNS) and *Mammaliicoccus* species isolated from broiler chickens. Increasing evidence suggests that non-*aureus* staphylococci are emerging as important reservoirs of antimicrobial resistance genes within livestock environments, posing potential risks for both animal health and zoonotic transmission (Szafraniec et al. 2020, Silva et al. 2023) (Yilmaz et al. 2017).

In this study, 108 isolates were identified through 16S rRNA sequencing, representing two *Mammaliicoccus* species and eleven different CoNS species (Supplementary file). These findings are consistent with previous reports indicating high species diversity of staphylococci in poultry production systems. A total of eight samples such as *M. sciuri*, *M. lentus*, *S. arlettae*, *S. shini*, *S. gallinarum* and *S. simulans* (KB08, KB10, ALBM18, M1, M2, C2, MH4 and POL29) were selected on the basis of the 16S rRNA results for Whole genome sequencing and the obtained sequences were deposited in the NCBI Genome database with the following accession numbers: GCA_039655975.1, GCA_050106305.1, GCA_050106295.1, GCA_051337605.1, GCA_039655755.1, GCA_038901535.1, GCA_038901555.1 and GCA_050106335.1.

The previous study (Szafraniec et al. 2025) reported multiple CoNS species associated with skeletal infections in broiler chickens, including *S. cohnii*, *S. epidermidis*, *S. hominis*, *M. lentus*, *S. saprophyticus*, and *S. gallinarum*. Similarly, studies have demonstrated that *M. sciuri* and *S. simulans* frequently occur in livestock and may serve as reservoirs of resistance determinants that can disseminate across bacterial populations (Rahman et al. 2026).

In pan-genome analysis, the clustered protein-coding genes into core genes (conserved in related groups of genomes) (Segata et al. 2011) and singleton families (unique to individual genomes) arose via horizontal gene transfer (Babalola et al. 2026). In our study, all eight species have large genomes and a high rate of horizontal gene transfer revealed as an open pan-genome (Rouli et al. 2015). OrthoVenn 3 analysis, among all eight species 1611 orthologous gene clusters originate from a common ancestor (Remm et al 2001). In plasmid identification, multiple plasmid replication (*rep*) genes were present indicating plasmid diversity and a wide range of mobile genetic elements that facilitate genetic adaptation. The families such as *rep13*, *rep7a*, *rep20*, and *rep10* were associated with specific resistance genes (*str*, *cat*, *blaZ*, *erm(C)*), *rep19* associated with *blaZ*, *tet(K)*, *rep 20/21* associated with *blaZ*, *qacA* and exhibited widespread horizontal transfer, *rep22* was associated with bleomycin, aminoglycosides and heavy metals (Contarin et al. 2014) (Egyir et al. 2022). The replicons *rep10* and *rep7a* with high nucleotide identities (99–100%) are highly conserved and may represent common plasmid backbones in all eight species. The isolates such as M2 (*S. gallinarum*), POL-29 (*S. arlettae*), KB08 (*M. lentus*), and KB-10 (*S. shini*) carry *rep13*, *rep19c*, *rep20*, *rep21*, *repUS43*, and *rep22* indicating the coexistence of multiple compatible plasmids within the same bacterial host. The plasmid replicon typing scheme was developed by (Jensen et al. 2010) for *Staphylococcus* spp. and identified harbor multiple plasmid replication genes, reflecting extensive plasmid exchange and diversity. The large staphylococcal multiresistance plasmids possess antimicrobial resistance, evolutionarily related replication systems and other adaptive traits through horizontal gene transfer (Firth et al. 2000).

Staphylococcus spp. may serve as an important reservoir of diverse plasmids, the spread of mobile genetic elements and contribute to bacterial adaptation based on these findings. In this study the *Siphoviridae* family prophage sequences were identified among *Staphylococcus* spp., and it is reported in a previous study (Oliveira et al. 2019). Another study demonstrated prophage plays an important role in genome evolution, horizontal gene transfer, and the dissemination of virulence and antimicrobial resistance determinants among *Staphylococcus* spp. (Xia & Wolz 2014; Haaber et al. 2017). Whereas prophage-associated genes were not found in *Mammaliicoccus* spp. may reflect species-specific differences in prophage acquisition although the identification of prophages in large numbers of isolates is needed to confirm this observation. The MGE-associated genes were highly abundant among all the isolates in MobileOG-db analysis indicating strong potential for horizontal gene transfer. (Partridge et al. 2018) highlighted that MGEs are crucial for the spread of antimicrobial resistance and drivers of bacterial genome evolution. The CRISPR sequences were identified in all eight species indicating distinct adaptive immune strategies within Staphylococcal and Mammaliicoccal lineages. In *Staphylococcus simulans*, CRISPR-associated Cas proteins such as Cas1, Cas2, and Cas9 were observed, signifying a fully functional Type II CRISPR system, where Cas9 mediates sequence-specific endonuclease cleavage and Cas1–Cas2 drive spacer acquisition (Cruz-Lopez et al. 2021). The Cas3 protein responsible for

degradation by helicase or nuclease enzymes present in *S. shini*, *S. arlettae*, *S. gallinarum*, and *M. sciuri* indicates operational Type I systems. The absence of the Cas protein *M.lentus* may indicate limited horizontal gene transfer by blocking phage infection and plasmid conjugation (Marraffini & Sontheimer 2008). Whole-genome sequencing revealed an extensive repertoire of antimicrobial resistance determinants. A total of 133 AMR genes were detected, with 26 genes consistently present across all 8 sequenced species. Most of the AMR genes family belongs to major facilitator superfamily (MFS), small multidrug resistance (SMR), ATP-binding cassette (ABC) antibiotic efflux pump, and glycopeptide resistance gene cluster.

In conclusion there are several antimicrobial resistance genes were present in Coagulase-negative *Staphylococcus* and *Mammaliococcus* spp. These genes confer resistance to multiple antibiotic classes through diverse mechanisms including antibiotic inactivation, target modification, target protection, and active efflux. The presence of multiple resistance genes highlights the genetic plasticity of staphylococcal genomes and their capacity to accumulate resistance determinants through horizontal gene transfer mediated by mobile genetic elements such as plasmids and transposons.

Declarations

Competing interests

Authors declare that they have no competing interests.

Ethical approval

The study was conducted at a commercial poultry farm with required permissions and ethical clearances. Ref no. SF/12052025.

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Author Contribution

V.T.G. conducted the experiments, carried out sequence data analysis, and molecular docking analysis and wrote the manuscript. K.R. collected the samples. R.U. and J.M. supervised the work, made the experimental design and reviewed the manuscript.

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Data availability statement

The genomic data have been deposited in GenBank (NCBI), and the corresponding accession numbers GCA_039655975.1, GCA_050106305.1, GCA_050106295.1, GCA_051337605.1, GCA_039655755.1, GCA_038901535.1, GCA_038901555.1 and GCA_050106335.1. All other data are presented in the manuscript and supplementary file.

References

1. Arkin AP, Cottingham RW et al (2018). KBase: the United States department of energy systems biology knowledgebase. *Nature biotechnology*, 36(7), 566–569. <https://doi.org/10.1038/nbt.4163>
2. Babalola OO, Yousuf S, Adefiranye OO, Chukwuneme CF, Ojuederie OB, Adeniji AA (2026) In silico Screening of Biosynthetic Traits Driving Biotechnological Utility of Coagulase Negative, Food-Associated *Staphylococcus* Strains. *The Microbe*, 100732. <https://doi.org/10.1016/j.microb.2026.100732>
3. Bankevich A, et al (2012) SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of computational biology*. 19, 455–477. <https://doi.org/10.1089/cmb.2012.0021>
4. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
5. Caputo A, Fournier PE, Raoult D (2019) Genome and pan-genome analysis to classify emerging bacteria. *Biology direct*, 14(1), 5. <https://doi.org/10.1186/s13062-019-0234-0>
6. Carattoli A, Zankari E et al (2014) In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrobial agents and chemotherapy*, 58(7), 3895–3903. <https://doi.org/10.1128/aac.02412-14>
7. Contarin R, Murri S, Drapeau A, Cayssials T, Madec JY, Dordet-Frisoni E, Haenni M (2025) Comprehensive genomic analysis of antibiotic resistance plasmids in animal-associated *Staphylococcus aureus* in France. *Microbiology Spectrum*, 13(10), e00772-25.

8. Cruz-Lopez EA, et al. (2021) Identification and Characterization of the CRISPR/Cas System in *Staphylococcus aureus* Strains From Diverse Sources. *Frontiers in Microbiology*. <https://doi.org/10.3389/fmicb.2021.656996>
9. Egyir B, Dsani E, Owusu-Nyantakyi C, Amuasi, GR, Owusu FA, Allegye-Cudjoe E, Addo KK (2022) Antimicrobial resistance and genomic analysis of staphylococci isolated from livestock and farm attendants in Northern Ghana. *BMC microbiology*, 22(1), 180. <https://doi.org/10.1186/s12866-022-02589-9>
10. Firth N, Apisiridej S, Berg T, ORourke BA, Curnock S, Dyke KGH, Skurray RA (2000) Replication of staphylococcal multiresistance plasmids. *Journal of Bacteriology*, 182(8), 2170–2178. <https://doi.org/10.1128/jb.182.8.2170-2178.2000>
11. Haaber J, Penadés JR, Ingmer H (2017) Transfer of antibiotic resistance in *Staphylococcus* by bacteriophages. *mBio*. 8(6):e01725-17. <https://doi.org/10.1016/j.tim.2017.05.011>
12. Jensen LB, Garcia-Migura L, Valenzuela AJ, Løhr M, Hasman H, Aarestrup FM (2010) A classification system for plasmids from enterococci and other Gram-positive bacteria. *Journal of Microbiological Methods*, 80(1), 25–43. <https://doi.org/10.1016/j.mimet.2009.10.012>
13. Johansson MH, Aarestrup FM, Petersen TN (2023) Importance of mobile genetic elements for dissemination of antimicrobial resistance in metagenomic sewage samples across the world. *Plos one*, 18(10), e0293169. <https://doi.org/10.1371/journal.pone.0293169>
14. Johansson MH, Petersen TN et al (2025) Investigation of mobile genetic elements and their association with antibiotic resistance genes in clinical pathogens worldwide. *PloS one*, 20(8), e0330304. <https://doi.org/10.1371/journal.pone.0330304>
15. Kandasamy S, Lee KH et al (2024) Whole genome sequencing of *Lactocaseibacillus casei* KACC92338 strain with strong antioxidant activity, reveals genes and gene clusters of probiotic and antimicrobial potential. *Frontiers in Microbiology*, 15, 1458221. <https://doi.org/10.3389/fmicb.2024.1458221>
16. Kim Y, Gu C, Kim HU, Lee SY (2020) Current status of pan-genome analysis for pathogenic bacteria. *Current opinion in biotechnology*, 63, 54–62. <https://doi.org/10.1016/j.copbio.2019.12.001>
17. Marraffini LA, Sontheimer EJ (2008) CRISPR Interference Limits Horizontal Gene Transfer in *Staphylococci* by Targeting DNA. *Science*, 322(5909), 1843–1845. <https://doi.org/10.1126/science.116577>
18. Najar IN, Sharma P, Das R et al(2025) Unveiling the probiotic potential of the genus *Geobacillus* through comparative genomics and in silico analysis. *Scientific Reports*, 15(1), 33748. <https://doi.org/10.1038/s41598-025-98392-3>
19. Oliveira H, Sampaio M, Melo LDR et al. (2019) *Staphylococci* phages display vast genomic diversity and evolutionary relationships. *BMC Genomics*. 20:357. <https://doi.org/10.1186/s12864-019-5647-8>
20. Partridge SR, Kwong SM, Firth N, Jensen SO (2018) *Mobile genetic elements associated with antimicrobial resistance*. *Clinical Microbiology Reviews*. 31(4):e00088-17. <https://doi.org/10.1128/cmr.00088-17>
21. Rahman MH et al (2026) Antimicrobial resistance profiles of non-aureus *Staphylococci* isolated from farm animals, farm environments and companion animals. *Sci Rep* 16, 5564 (2026). <https://doi.org/10.1038/s41598-026-36455-9>
22. Ramesh V, Sivakumar R, Annamanedi M, Chandrapriya S, Isloor S, Rajendhran J, Hegde NR (2024) Genome sequencing and comparative genomic analysis of bovine mastitis-associated non-aureus staphylococci and mammaliococci (NASM) strains from India. *Scientific Reports*, 14(1), 29019. <https://doi.org/10.1038/s41598-024-80533-9>
23. Remm M, Storm CE, Sonnhammer EL (2001) Automatic clustering of orthologs and in-paralogs from pairwise species comparisons. *Journal of molecular biology*, 314(5), 1041–1052. <https://doi.org/10.1006/jmbi.2000.5197>
24. Rouli L, Merhej V, Fournier PE, Raoult D (2015) The bacterial pangenome as a new tool for analysing pathogenic bacteria. *New microbes and new infections*, 7, 72–85. <https://doi.org/10.1016/j.nmni.2015.06.005>
25. Schindler BD, Kaatz GW (2016) Multidrug efflux pumps of Gram-positive bacteria. *Drug Resistance Updates* 27 1–13. <https://doi.org/10.1016/j.drug.2016.04.003>
26. Seemann T (2014) Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>
27. Segata, N, Huttenhower C (2011) Toward an efficient method of identifying core genes for evolutionary and functional microbial phylogenies. *PloS one*, 6(9), e24704. <https://doi.org/10.1371/journal.pone.0024704>
28. Silva V, Araújo S, Monteiro A, Eira J, Pereira JE, Maltez L, Igrejas G, Lemsaddek TS, Poeta P (2023) *Staphylococcus aureus* and MRSA in livestock: antimicrobial resistance and genetic lineages. *Microorganisms*, 11(1), 124. <https://doi.org/10.3390/microorganisms11010124>
29. Sun J, Lu F, Luo Y, Bie L, Xu L, Wang Y (2023) OrthoVenn3: an integrated platform for exploring and visualizing orthologous data across genomes. *Nucleic acids research*, 51(W1), W397-W403. <https://doi.org/10.1093/nar/gkad313>
30. Szafraniec GM, Chrobak-Chmiel D, Dolka I, Adamczyk K, Sulecki K, Dolka B (2025) Virulence factors and biofilm forming ability of *Staphylococcus* species isolated from skeletal lesions of broiler chickens. *Scientific Reports* 15(1) 10807 <https://doi.org/10.1038/s41598-025-95006-w>
31. Szafraniec GM, Szeleszczuk P, Dolka B (2020) A review of current knowledge on *Staphylococcus* agent in poultry. *Animals* 10(8) 1421. <https://doi.org/10.3390/ani10081421>
32. Szafraniec GM, Szeleszczuk P, Dolka B (2022) Review on skeletal disorders caused by *Staphylococcus* spp. in poultry. *Veterinary Quarterly* 42(1) 21–40. <https://doi.org/10.1080/01652176.2022.2033880>

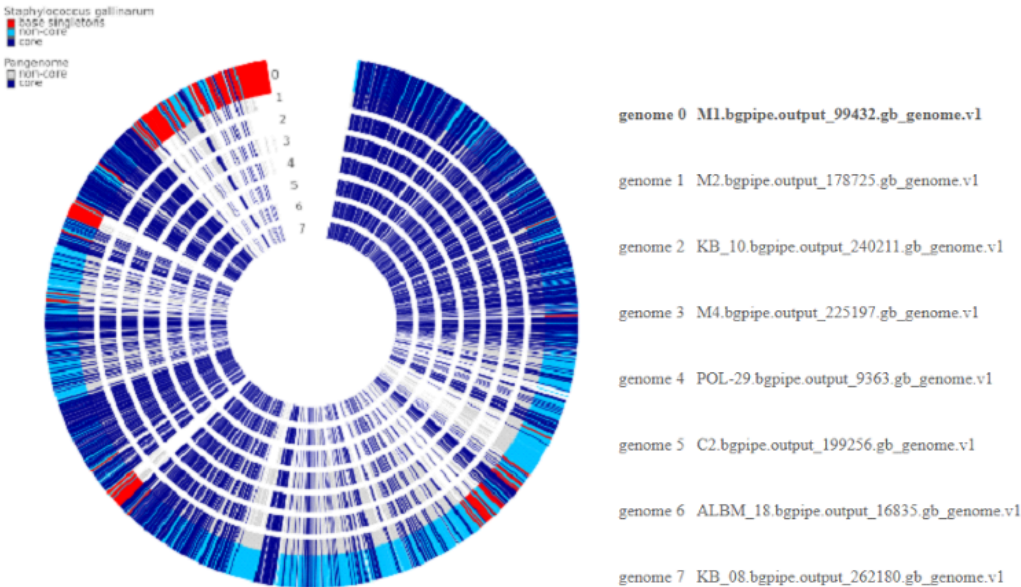
33. Tao S, Chen H, Li N, Liang W (2022) The Application of the CRISPR-Cas System in Antibiotic Resistance. *Infection and Drug Resistance*, 15, 4155–4168. <https://doi.org/10.2147/IDR.S370869>

34. Xia G, Wolz C (2014) Phages of *Staphylococcus aureus* and their impact on host evolution. *Infection, Genetics and Evolution*. 21:593–601. 10.1016/j.meegid.2013.04.022

35. Yılmaz EŞ, Aslantaş O (2017) Antimicrobial resistance and underlying mechanisms in *Staphylococcus aureus* isolates. *Asian Pacific journal of tropical medicine* 10(11) 1059–1064. [https://doi.org/10.1016/S0378-1135\(00\)00197-8](https://doi.org/10.1016/S0378-1135(00)00197-8)

Figures

a



b

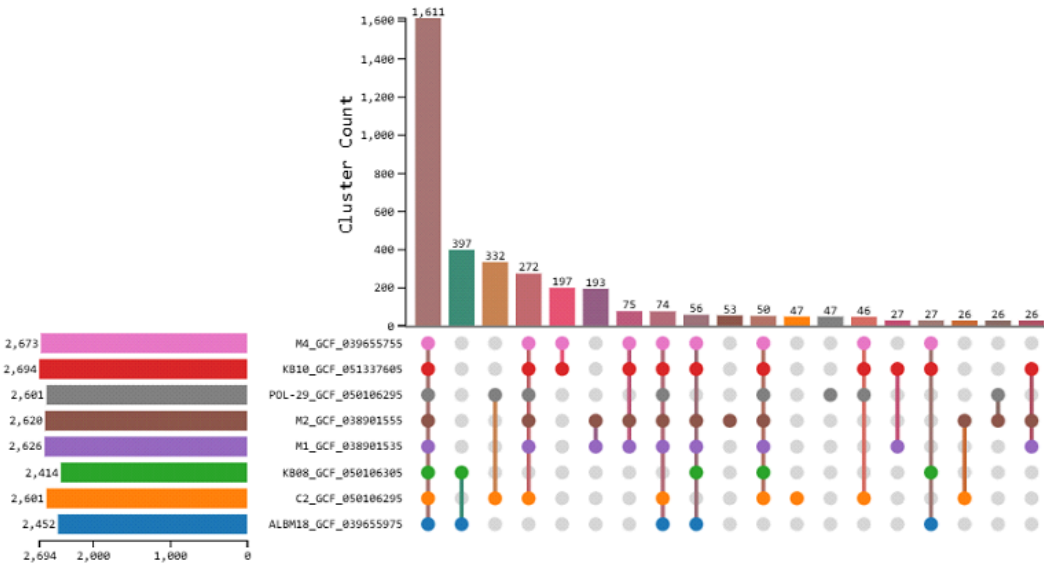


Figure 1

Pangenome analysis of the CoNS and *Mammaliicoccus* species (a) Pangenome circle plot displaying core, non-core, and singleton protein families; (b) UpSet plot using OrthoVenn 3 representing unique and shared orthologous clusters, with the left bars showing clusters per species and the right bars indicating shared clusters among species.

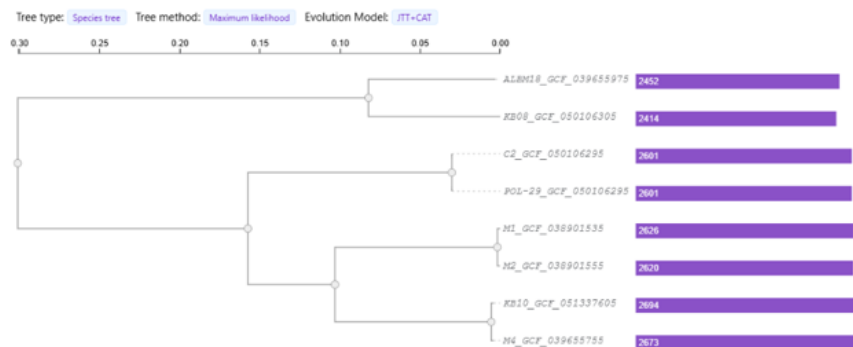


Figure 2

Phylogenetic relationships among the *Staphylococcus* and *Mammaliococcus* spp. based on OrthoVenn3 analysis. Phylogenetic tree representing a high degree of genomic similarity and share a larger proportion of conserved orthologous gene families.



Figure 3

Distribution of mobile genetic elements (MGEs) identified in CoNS and *Mammaliococcus* spp. Donut charts represent the abundance of different MGE categories, including insertion elements (IE), repeat regions (RRR), prophages (P), sequence transposons/domains (STD), and transposons (T), with values indicating the number of elements detected in each isolate.

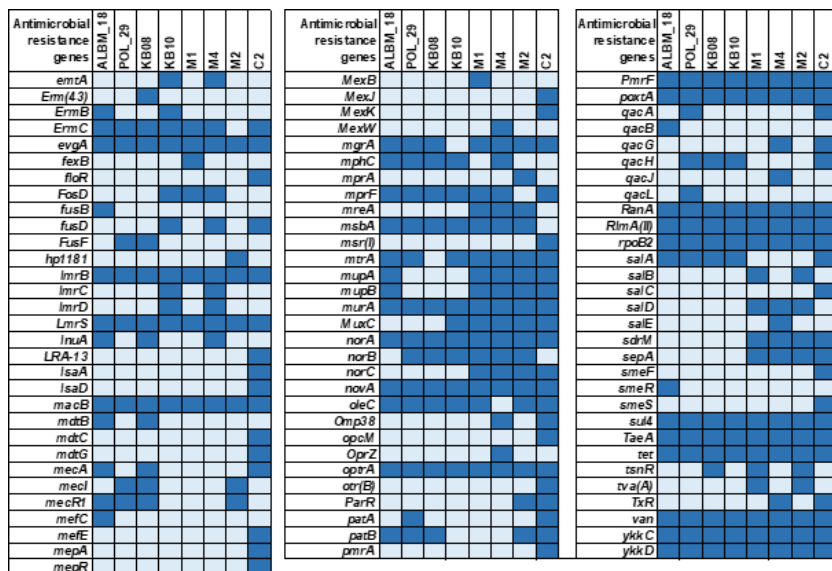


Figure 4

Heatmap showing the distribution of antimicrobial resistance (AMR) genes among the eight bacterial isolates. Rows represent individual AMR genes, while columns correspond to the analyzed isolates. Dark blue represents the presence of gene and light blue represent the absence of gene. The heatmap highlights the variation in AMR gene profiles across the isolates.

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