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# Genomic features of mecA-harboring Staphylococcus epidermidis infecting an illegally traded rufous-bellied thrush (T. rufiventris)

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**Case Report**

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## Abstract

*Staphylococcus epidermidis* is an opportunistic bacterium frequently involved in healthcare-associated infections and has become increasingly relevant in One Health when associated with methicillin resistance profile. Despite its significance in human medicine, the circulation of *S. epidermidis* in wildlife still poorly reported. In this study, we communicate the isolation and genomic characterization of a multidrug-resistant *S. epidermidis* Sequence Type 280 isolated from a skin lesion in an illegally traded wild rufous-bellied thrush (*Turdus rufiventris*). Antimicrobial susceptibility tests and genome sequencing revealed extensive multidrug resistance, including resistance to methicillin, related to the presence of antimicrobial resistance (*mecA*; *blaZ*; *aadD*; *aac(6') – aph(2"); fosB; erm(C)* and *rpoB* (D471E/I527M)). This finding provides compelling evidence for the global dissemination and ecological plasticity of this clone. Ultimately, this synanthropic sentinel's death related to a methicillin resistant *S. epidermidis* emphasizes the impact that urbanization has on antibiotic resistance, highlighting the need to incorporate wildlife monitoring into One Health surveillance models.


## 1. BACKGROUND

The fragmentation of natural habitats caused by the rapid pace of urbanization has intensified the interaction between humans, domestic animals, and wildlife, facilitating the transmission of resistance genes because of the creation of ecological corridors (Doyle et al. 2025). In urbanized environments, synanthropic birds are considered vital epidemiological sentinels, as they reflect the antimicrobial selection pressure existing in shared ecosystems. The presence of antimicrobial-resistant strains in wild animals indicates that, while these animals may serve as sentinels, they also may play a role as mobile reservoirs that facilitate the dissemination of multidrug-resistant lineages across ecological boundaries (Vittecoq et al. 2016).

The Rufous-bellied Thrush (*Turdus rufiventris*) is a non-migratory resident species widely distributed across South America, occurring in southern Bolivia, Paraguay, Uruguay, central and coastal Argentina, and southern Brazil (Collar 2020). Although it inhabits a variety of natural environments, including gallery forests, Cerrado woodlands, and the Pampas, this species is highly tolerant of urbanized landscapes, which provide favorable conditions for colonization and population growth (Vazquez et al. 2024).

In human-animal-environment, methicillin-resistant non-aureus staphylococci species are becoming increasingly important as well(Nemeghaire et al. 2014; Schauer et al. 2021) as *Staphylococcus aureus*, which has been historically prioritized. The rise of these resistant strains, particularly *Staphylococcus epidermidis*, demonstrates the need for surveillance across diverse ecological niches, given that these are important reservoirs and vectors for bacterial resistance determinants (Dolejska and Literak 2019).

Among the non-aureus staphylococci, *S. epidermidis* has emerged as a significant opportunistic pathogen, distinguished by its genomic plasticity and ability to acquire several antimicrobial resistance (AMR) genes (Edslev et al. 2025b). While this bacterium is usually associated with infectious processes in humans, the circulation of high-risk lineages (Kellgren et al. 2024), such as Sequence Type 280 (ST280), in non-humans remains understudied.

This study reports the isolation and genomic characterization of an *S. epidermidis* ST280 strain with multiple antimicrobial resistance genes found in a skin lesion of an rufous-bellied thrush (*T. rufiventris*) at a wildlife rehabilitation center in São Paulo, Brazil. Additionally, to the best of our knowledge, this is the first reported case in which *S. epidermidis* is associated directly with the death of a wild animal. The identification of the resistance mechanisms present in this strain, including genes conferring resistance to beta-lactams, aminoglycosides, rifampicin and macrolides, highlights the threat that bacterial clones pose to urban-wildlife interactions and underscores the urgent need for genomic surveillance in free-living species.

## 2. CASE PRESENTATION

On September 2024, an illegally traded rufous-bellied thrush *(Turdus rufiventris)* was rescued by the municipal guard and admitted in a wild animal medical care and rehabilitation center in São Paulo State, Brazil. The animal presented skin lesions in cephalic region and a swab sample was collected for microbiological diagnostic. The swab was later streaked on Blood Agar (5% v/v) and incubated at 37°C overnight. White circular colonies were identified as *Staphylococcus epidermidis* by MALDI-TOF. After unsuccessful treatment with cefalexin (0.08 mg/g of body weight) and ketoconazole (0.02 mg/g of body), and worsening of its clinical status, the animal was euthanized.

The SL01 antimicrobial resistance profile was assessed by the minimum inhibitory concentration (MIC) values of the antimicrobials, which were determined by the broth microdilution method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100-S36 guidelines (CLSI, 2026). The antimicrobial concentration range tested is presented in Table 1.

The genomic library was prepared with 300 ng of dsDNA using a modified protocol of Illumina COVIDSeq (Illumina Inc., San Diego, USA), starting from the tagmentation step, and all subsequent procedures were performed according to the manufacturer's instructions. Library quality was assessed using the TapeStation High Sensitivity D1000 ScreenTape Assay (Agilent Technologies, Santa Clara, USA). The whole genome sequencing of the SL01 isolate (Genbank Accession: JBWXTE000000000) was performed using the MiSeq i100 platform (Illumina Inc., San Diego, CA) with 150 bp single-end reads. The quality of the reads was assessed by FastQC (v.0.12.1) (https://github.com/s-andrews/FastQC), and the reads were trimmed using Trimmomatic (v.0.39) (https://github.com/usadellab/Trimmomatic).

Unicycler (v.0.5.1) (https://github.com/rrwick/Unicycler) was used to assemble the draft genome, which was later annotated with Prokka (v.1.14.6) (https://github.com/tseemann/prokka). Genomic data were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Antimicrobial resistance genes were screened by the ABRicate v1.0.1 platform (https://github.com/tseemann/abricate) with ResFinder 4.6.0 (http://genepi.food.dtu.dk/resfinder). Later, bacterial typing tools were used to identify Multi-Locus Sequence Typing (MLST) (MLST v.2.0) (https://github.com/tseemann/mlst).

All *Staphylococcus epidermidis* genomes available in NCBI's Genome platform (https://www.ncbi.nlm.nih.gov/home/genomes/) were compared to SL01 genome using FastANI v1.32 (https://github.com/ParBLiSS/FastANI). The 50 genomes with the highest ANI were selected for phylogenetic analysis. The genome annotation was performed using Prokka v1.14.6 (https://github.com/tseemann/prokka), the core orthologous genes of these genomes were concatenated and aligned using Roary (v3.13.0) (https://github.com/sanger-pathogens/Roary), and a maximum likelihood phylogenetic tree was constructed with IQ-TREE (v2.4.0) (https://iqtree.github.io/) (ultrafast bootstrap 1000 x). For comparative analysis, a phylogenomic tree and a heatmap with antimicrobial resistance genes were constructed with R software (R Core Team 2021) by RStudio (RStudio Team 2020) using the ggplot2 (Wickham 2009) and ggtreeExtra (Xu et al. 2021) packages for graphing.

The isolate obtained from the skin lesion found in a rufous-bellied thrush was identified as *Staphylococcus epidermidis*. The Minimum Inhibitory Concentration (MIC) test found that the SL01 strain exhibited resistance to multiple antimicrobial classes (Table 1). The isolate showed elevated resistance to beta-lactams, specifically penicillin (MIC = 0.5 µg/mL) and oxacillin (MIC = 1 µg/mL), as well as to gentamicin (MIC = 16 µg/mL). Additionally, resistance was observed for rifampicin (MIC = 8 µg/mL), and clindamycin (MIC = 8 µg/mL).

Table 1
Antimicrobial susceptibility profile and corresponding antimicrobial resistance genes for *Staphylococcus epidermidis* SL01 strain results and respective antimicrobial resistance genes associated.

| **Antimicrobial** | **Minimum inhibitory concentration (µg/mL)** | **Minimal concentration tested (µg/mL)** | **Maximum concentration tested (µg/mL)** | **Result (CLSI, 2026)** | **Antimicrobial resistance gene related** |
|---|---|---|---|---|---|
| Oxacillin | 1 | 0.25 | 2 | Resistant | *mecA* |
| Penicillin | 0.5 | 0.0625 | 1 | Resistant | *mecA; blaZ* |
| Gentamicin | 16 | 2 | 32 | Resistant | *aadD; aac(6') – aph(2")* |
| Chloramphenicol | 2 | 2 | 64 | Susceptible | - |
| Ciprofloxacin | 1 | 0.5 | 8 | Susceptible | - |
| Clindamycin | 8 | 0.25 | 8 | Resistant | *erm(C)* |
| Rifampicin | 8 | 0.5 | 8 | Resistant | *rpoB* (D471E/I527M) |
| Sulfamethoxazole/trimethoprim | 38/2 | 19/1 | 152/8 | Susceptible | - |
| Vancomycin | 2 | 2 | 64 | Susceptible | - |
| Tetracycline | 2 | 2 | 32 | Susceptible | - |

The multi-locus sequencing typing analysis revealed that the *S. epidermidis* isolate belonged to ST280. Annotation analysis revealed D471E/I527M substitutions in subunit of bacterial beta-subunit RNA polymerase. The ABRicate screening identified the presence of genes conferring resistance to β-lactams (*mecA*; *blaZ*), aminoglycosides (*aadD*; *aac(6´)-aph(2")*), fosfomycin (*fosB*), and macrolides (*erm(C)*).

Phylogenomic and epidemiological analysis reveals that *S. epidermidis* ST280 is distributed worldwide. Most of *S. epidermidis* strains (90.2%; 46/51) were reported in humans and showed antimicrobial resistance encoding genes for aminoglycosides (74.5%; 38/51), beta-lactams (94.1%; 48/51) and fosfomycin (100%) (Fig. 1). All studied samples carried the fosfomycin resistance gene *fosB*, 46 samples (90.2%; 46/51) were positive for *mecA* and 26 (51.0%; 26/51) positive for *blaZ*. SL01 isolate did not cluster with any specific pattern of strains (Fig. 1).

# 3. DISCUSSION

In this study we reported the isolation of a multidrug-resistant (MDR) *Staphylococcus epidermidis* ST280 from a fatal skin lesion in a rufous-bellied thrush (*Turdus rufiventris*). This finding is particularly significant as *S. epidermidis* is a well-documented opportunistic pathogen associated with hospital environments and healthcare-associated infections in humans (Rupp 2014; Dong et al. 2018; Chabi and Momtaz 2019; Martínez-Santos et al. 2022; Demiselle et al. 2023) and demonstrates the strong impact that anthropogenic pressure has on wildlife health.

SL01 isolate genomic profile reveals a strict relation to the animal outcome, considering its difficulty in its pharmacological treatment. The presence of the *mecA* and *blaZ* genes are related to the lack of susceptibility to beta-lactams. In addition, the identification of *erm(C)*, *aadD*, and *aac(6')-aph(2")* genes highlights the strain's ability to resist multiple classes of antimicrobials, including macrolides and aminoglycosides. In a healthcare context, the multiple antimicrobial resistance demonstrated in this strain shows a clinical challenge in treatment (Kalın et al. 2023), which may have contributed to treatment failure.

The identification of the SL01 isolate in a wild bird in Brazil provides compelling evidence for the global dissemination of ST280 *S. epidermidis* lineages (Miragaia et al. 2008; Lee et al. 2018). This finding suggests that wild birds may act as environmental sentinels, bringing together clinical settings, livestock production, and natural ecosystems (Dalazen et al. 2023; Mbuthia and Hoza 2025). The genomic resemblance between SL01 and isolates recovered over a decade ago in different parts of the world (Fig. 1) indicates a successful clone with remarkable ecological plasticity (Pitout and DeVinney 2017). In addition, this intercontinental and multi-host connectivity points towards human-to-animal transmission, a condition where human-associated pathogens spill over into wildlife because of environmental contamination (Ellwanger et al. 2022). From a One Health perspective, our results highlight that the virulence potential of *S. epidermidis* is not confined to hospitals, but is actively circulating across the human-animal-environment interface, regardless of geographical borders (Persson Waller et al. 2025).

To the best of our knowledge, this is the first case linked to an MDR *S. epidermidis* infection to a wild animal's demise, marking a significant turning point in wildlife pathology. The skin lesion in *T. rufiventris* with a methicillin-resistant *S. epidermidis* shows that these human-associated lineages can transcend species boundaries and may exhibit pathogenic potential in susceptible wild hosts, in contrast to earlier research that classified non-aureus staphylococci in wildlife as commensals or asymptomatic carriage (González-Martín et al. 2020). Since fosfomycin and rifampicin are frequently used as a last resort for MDR staphylococcal infections (Dinh et al. 2012; Bodmann et al. 2025), the presence of *rpoB* (D471E/I527M) mutation, substitution frequently associated with rifampicin resistance in *Staphylococcus epidermidis* (Edslev et al. 2025a) and fosfomycin resistance (*fosB*) further complicates the resistance landscape. Therefore, these findings support the need to incorporate wildlife monitoring into One Health models (Barroso et al. 2023).

The death of a synanthropic sentinel due to an MDR clone like ST280 serves as a stark indicator of "antimicrobial pollution" in urban centers like São Paulo (Laborda et al. 2022). Future surveillance must focus not only on the presence of these bacteria but also on their pathological impact, as the transmission of hospital-associated resistance determinants to wildlife may lead to localized biodiversity losses and create permanent environmental reservoirs of high-risk pathogens. Since the spread of hospital-associated resistance determinants to wildlife may result in localized biodiversity losses and establish permanent environmental reservoirs of high-risk pathogens (Laborda et al. 2022; Silva et al. 2025), future surveillance must concentrate on both the presence of these bacteria and their pathological impact.

# Declarations


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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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## Figures

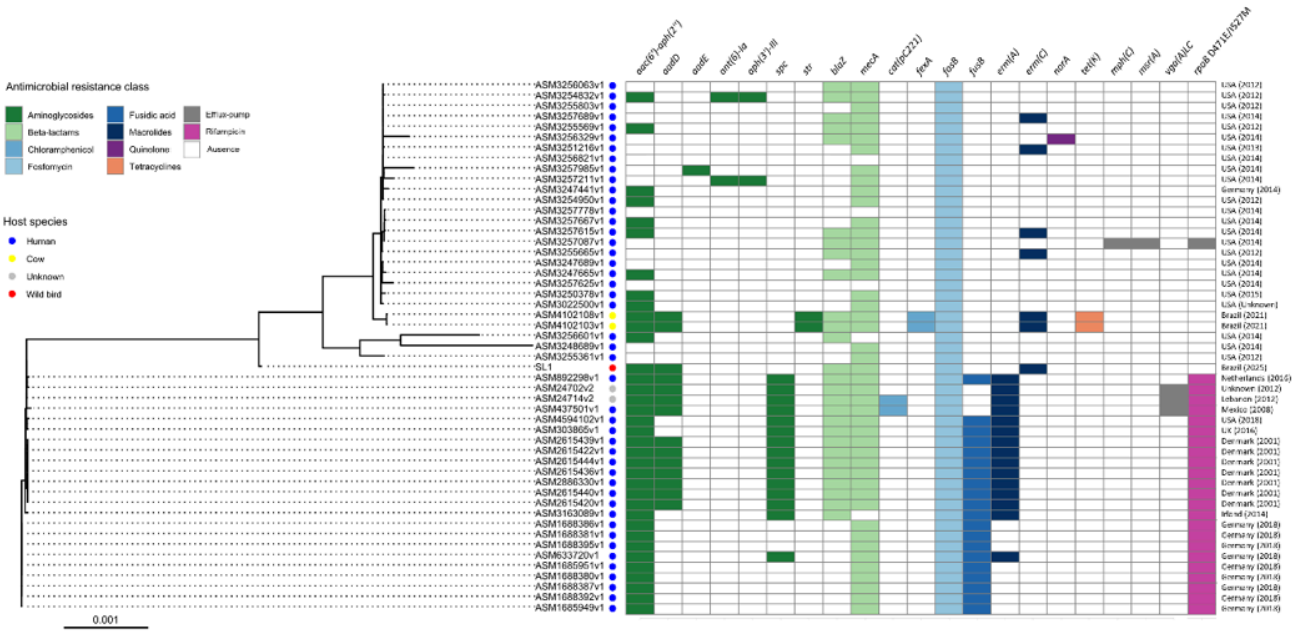



**Figure 1**

Phylogenetic and comparative genomic analysis of *Staphylococcus epidermidis* isolates. Maximum likelihood phylogenetic tree based on the core genome (concatenated orthologous genes) of *S. epidermidis* SL01 and the 50 genomes with the highest Average Nucleotide Identity (ANI) from the NCBI database. The tree was constructed using IQ-TREE with 1,000 ultrafast bootstrap replicates. The associated heatmap displays the presence (filled) and absence (empty) of antimicrobial resistance genes identified via Prokka annotation. The analysis was visualized using R software with *ggplot2* and *ggtreeExtra*packages.

## Supplementary Files

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- AnimalSampleConcession.pdf