

First report of *Tabanus (Tabanus) rubidus* Wiedemann, 1821 (Diptera, Tabanidae) from Assam, India, with detection of multidrug-resistant *Mammaliicoccus sciuri* and *Staphylococcus kloosii*

Chanda Gowala¹, Mayoushree Gogoi¹, Niraj Singh², Alok Kumar Srivastav³ and Abesh Chakraborty^{1*}

¹Department of Zoology, RSLSC, The Assam Royal Global University, Guwahati 781035, Assam, India.

²Department of Microbiology, RSBSC, The Assam Royal Global University, Guwahati 781035, Assam, India.

³Department of Health Science, University of the People, Pasadena, California 91101, United States.
Email: abeshc1@gmail.com*

ABSTRACT: A survey of *Staphylococcus* and *Mammaliicoccus* bacteria in *Tabanus (Tabanus) rubidus* Wiedemann, 1821 (Diptera, Tabanidae) was conducted in three habitats in Assam, India — Lokhrah, Ghar Chuk, and the Royal Global University campus — during peak fly activity in 2024. This constitutes the first record of *T. rubidus* from Assam. A total of 120 adult flies were collected and their morphological identity confirmed under a stereomicroscope using established taxonomic keys. Bacterial identification using the VITEK® 2 Compact system confirmed 168 staphylococcal isolates across six species. *Mammaliicoccus sciuri* was the most prevalent (82 isolates, 48.8%), followed by *Staphylococcus kloosii* (44 isolates, 26.2%). Antimicrobial susceptibility testing against 13 drugs revealed that 51.2 per cent of *M. sciuri* isolates were multidrug-resistant (MDR), with high resistance to methicillin (47.8%), erythromycin (65.4%), and tetracycline (40.5%). Among *S. kloosii*, 31.8 per cent were MDR, with notable resistance to penicillin (72.7%) and clindamycin (38.2%). All isolates remained susceptible to vancomycin and linezolid. These findings document the carriage of multidrug-resistant staphylococci in *T. rubidus* in Assam, suggesting that horse flies may serve as environmental reservoirs of antimicrobial resistance.

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KEY WORDS: Record, survey, staphylococcal isolates, reservoir, antimicrobial resistance

INTRODUCTION

Horse flies (Diptera, Tabanidae) are widespread blood-feeding insects reported as mechanical carriers of various bacterial pathogens (Mullens, 2024). *Tabanus (Tabanus) rubidus* Wiedemann,

1821, a large tabanid species with type locality in Bengal, has been recorded from West Bengal, Meghalaya, Orissa, Sikkim, and Arunachal Pradesh in India, and from China, Indonesia, Pakistan, and the Philippines (Banerjee *et al.*, 2020). The species is predominantly active between May–July and

* Author for correspondence

November–February and feeds on cattle, buffalo, horse, and wild herbivorous animals. It is closely related to *T. triceps* Thunberg, 1827, but is distinguished by the presence of a median stripe of pale tomentum on abdominal tergites I–VI appearing as a series of connected narrow triangles, and by characteristic step-like lateral abdominal stripes (Ricardo, 1911; Banerjee *et al.*, 2017). Despite its wide distribution in adjacent states, no published record of *T. rubidus* from Assam exists. This study reports *T. rubidus* for the first time from Assam and examines the staphylococcal bacteria harboured by this fly species.

Antimicrobial resistance (AMR) is a major global public health concern, and environmental reservoirs of resistant bacteria are increasingly recognized (Ferri *et al.*, 2017). *Mammaliicoccus sciuri* (formerly *Staphylococcus sciuri*) is an emerging opportunistic pathogen with high genetic adaptability and frequent multidrug resistance, recovered from livestock, wildlife, and clinical specimens (Boonchuay *et al.*, 2023). *Staphylococcus kloosii*, a coagulase-negative species, also carries resistance genes and has been found in diverse environmental contexts (Ocloo *et al.*, 2023). The occurrence of such bacteria in haematophagous insects is of public health interest because flies may acquire bacteria from their environment or from hosts during blood feeding. This study reports the presence and antimicrobial susceptibility profiles of staphylococci detected in *T. rubidus* from Assam both of which are first from the state.

MATERIALS AND METHODS

Adult *Tabanus* flies were collected from three locations in Assam, northeastern India, during June to September 2024 using sweep nets between 09:00 and 16:00 hours: Lokhrah (26.1944° N; 91.7697° E), an agricultural area with water bodies and livestock; Ghar Chuk (26.2156° N; 91.7845° E), a mixed farmland and residential zone; and the Royal Global University campus (26.2571° N; 91.5973° E), a semi-urban setting. A total of 120 adult flies were captured. Each specimen was placed individually in a sterile labelled 15mL tube, transported to the laboratory at 4°C, and identified

morphologically under a stereomicroscope using standard taxonomic keys for Tabanidae (Banerjee *et al.*, 2017).

All the 120 specimens collected in the survey were identified as *T. (T.) rubidus* on the basis of the following diagnostic characters: face covered with grey tomentum and white hairs; palpi stout and large, light yellow with black pubescence; frontal callus oblong with short linear extension; thorax dark reddish with grey tomentum and indistinct stripes; abdomen lilac to reddish brown, with median stripes composed of greyish-white tomentose triangular spots appearing as a series of connected narrow triangles on tergites I to VI; lateral stripes composed of greyish-white irregular tomentose spots appearing step-like; sides of abdomen serrated; femora dark; fore tibiae light yellowish but dark at apices; wings hyaline with a slight yellowish tinge in the middle. These characters distinguish *T. rubidus* from the related *T. triceps*, which lacks the series of connected triangular pale spots on the abdomen (Ricardo, 1911; Banerjee *et al.*, 2017).

For bacterial isolation, each fly was surface-sterilised by sequential immersion in 70 per cent ethanol (30 sec) and one per cent sodium hypochlorite (30 sec), followed by three one-minute rinses in sterile distilled water (Sharma *et al.*, 2020). Each sterilised fly was homogenised in 1mL phosphate-buffered saline (PBS, pH 7.4) and serially diluted. Aliquots were plated on, Nutrient Agar and incubated aerobically at 37°C for 24–48 hours. Gram-positive, catalase-positive cocci were presumptively classified as staphylococci and subjected to definitive species identification using the VITEK® 2 Compact system with GP identification cards (Funke and Funke-Kissling, 2005).

Antimicrobial susceptibility testing (AST) followed for 13 antibiotics across eight drug classes: penicillin G, oxacillin, and cefoxitin (beta-lactams); erythromycin and clindamycin (macrolides); gentamicin (aminoglycoside); tetracycline and doxycycline (tetracyclines); ciprofloxacin and levofloxacin (fluoroquinolones); trimethoprim-sulfamethoxazole (sulfonamide); vancomycin

(glycopeptide); and linezolid (oxazolidinone) (Jorgensen *et al.*, 2007). Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial classes (Magiorakos *et al.*, 2012). Quality control was maintained using *S. aureus* and *S. epidermidis*.

RESULTS AND DISCUSSION

A total of 120 specimens were morphologically confirmed as *T. (T.) rubidus* Wiedemann, 1821. This constitutes the first confirmed record of this species from Assam, India, extending its known distributional range in northeastern India beyond West Bengal, Meghalaya, Orissa, Sikkim, and Arunachal Pradesh (Maity *et al.*, 2020). The collected specimens exhibited body morphology, aligning with standard morphotaxonomic descriptions. The morphological identification was supported by the characteristic abdominal pattern of connected narrow triangular pale tomentose spots and step-like lateral stripes, which distinguishes it from *T. triceps*. The female-biased

collection reflects the haematophagous habit required for oogenesis in tabanids.

Microbiological processing of the flies yielded 168 staphylococcal isolates belonging to six species, confirmed via the VITEK® 2 Compact system (Table 1). This diversity suggests that *T. rubidus* harbours a complex bacterial community, mirroring findings from tabanid studies in other regions, such as northeastern China (Qiu *et al.*, 2024). *Mammaliicoccus sciuri* was the most prevalent species (48.8%), followed by *Staphylococcus kloosii* (26.2%). The high prevalence of *M. sciuri* (formerly *S. sciuri*) is significant, as it is an emerging opportunistic pathogen frequently associated with livestock and clinical environments (Boonchuay *et al.*, 2023). Other isolates included *S. xylosus* (11.9%), *S. epidermidis* (7.1%), *S. haemolyticus* (3.6%), and *S. saprophyticus* (2.4%).

Antimicrobial resistance (AMR) profiles revealed a high burden of resistance in the dominant species

Tabanus (Tabanus) rubidus as an Environmental Reservoir of MDR Bacteria in Assam, India

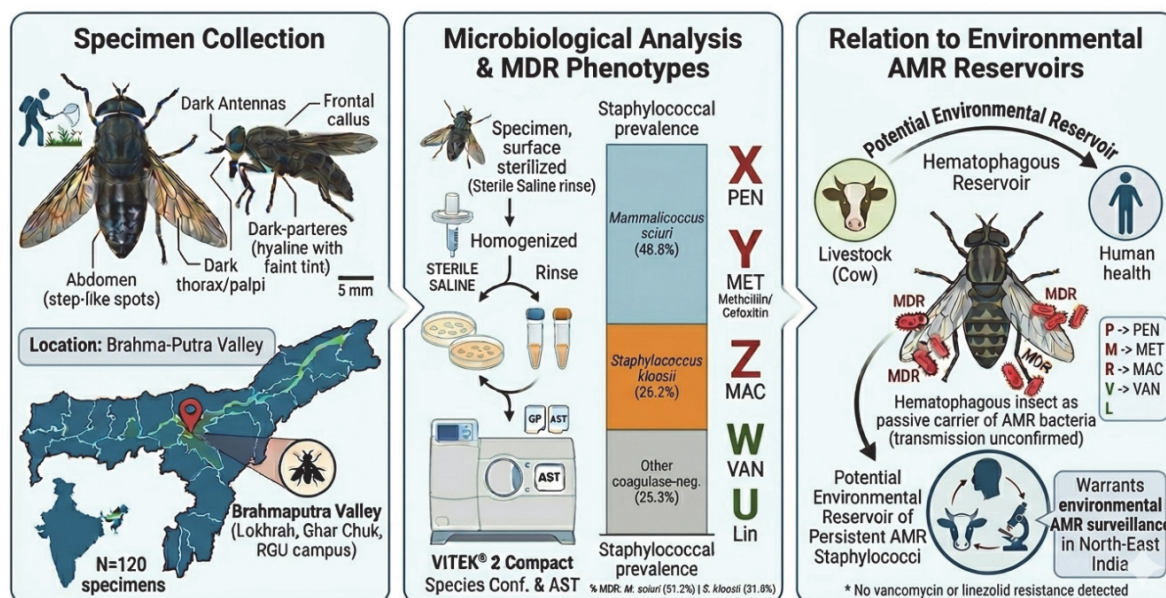


Fig. 1. Graphical summary of *Tabanus (Tabanus) rubidus* as a carrier of multidrug-resistant (MDR) bacteria in Assam, India, showing specimen collection sites in the Brahma-putra Valley, microbiological analysis pipeline (VITEK® 2 Compact), staphylococcal prevalence, key resistance phenotypes, and the potential role of the fly as an environmental reservoir of AMR staphylococci

Table 1. Distribution of *Staphylococcus* and *Mammaliicoccus* species isolated from *Tabanus (Tabanus) rubidus* (n = 168 isolates from 120 flies)

Species	No. of isolates (%)	Lokhrah	Ghar Chuk	RGU campus
<i>Mammaliicoccus sciuri</i>	82 (48.8)	34	24	24
<i>Staphylococcus kloosii</i>	44 (26.2)	16	14	14
<i>S. xylosus</i>	20 (11.9)	9	6	5
<i>S. epidermidis</i>	12 (7.1)	5	4	3
<i>S. haemolyticus</i>	6 (3.6)	3	2	1
<i>S. saprophyticus</i>	4 (2.4)	2	1	1
Total	168 (100)	69	51	48

Table 2. Antimicrobial resistance (% resistant) of *Mammaliicoccus sciuri* (n = 82) and *Staphylococcus kloosii* (n = 44).

Antibiotic	<i>M. sciuri</i> (n=82) %R	<i>S. kloosii</i> (n=44) %R
Penicillin G	68.3	72.7
Oxacillin (methicillin)	47.8	10.9
Cefoxitin	47.8	15.5
Erythromycin	65.4	15.5
Clindamycin	15.1	38.2
Gentamicin	18.0	20.0
Tetracycline	40.5	18.2
Doxycycline	38.5	16.4
Ciprofloxacin	22.9	12.7
Levofloxacin	20.0	10.9
TMP-SMX	25.4	15.5
Vancomycin	0.0	0.0
Linezolid	0.0	0.0

(Table 2). Among *M. sciuri* isolates, 47.8 per cent exhibited methicillin resistance (concurrent oxacillin and cefoxitin resistance). Resistance to penicillin G was 68.3 per cent, followed by erythromycin (65.4%) and tetracycline (40.5%). This high rate of methicillin resistance is a public health concern,

as it often confers co-resistance to most beta-lactam antibiotics. In *S. kloosii*, penicillin G resistance was the highest (72.7%), while methicillin resistance was lower at 15.5%. Reassuringly, all isolates remained susceptible to vancomycin and linezolid, consistent with their status as last-resort clinical treatments.

Multidrug resistance (MDR) was detected in 40.5 per cent of the total isolates, with a significantly higher prevalence in *M. sciuri* (51.2%), compared to *S. kloosii* (31.8%) (Table 3). These findings indicate that coagulase-negative staphylococci in haematophagous insects serve as important environmental reservoirs within the resistome (Ocloo *et al.*, 2023). While this study documents *T. rubidus* as a potential carrier of AMR

Table 3. Multidrug resistance (MDR) summary by species

Species	Total isolates	MDR isolates	MDR %
<i>Mammaliicoccus sciuri</i>	82	42	51.2
<i>Staphylococcus kloosii</i>	44	14	31.8
<i>S. xylosus</i>	20	7	35.0
<i>S. epidermidis</i>	12	3	25.0
<i>S. haemolyticus</i>	6	2	33.3
<i>S. saprophyticus</i>	4	0	0.0
Total	168	68	40.5

staphylococci, but it does not assess vector competence or demonstrate transmission nor provide evidence of vector competence or transmission to vertebrate hosts. The flies likely acquire these bacteria from soil, dung, or host skin during feeding (Greenberg, 1965). Given that northeastern India is a critical region for AMR monitoring (Malik *et al.*, 2024), these results highlight the necessity of incorporating insect-associated reservoirs into future surveillance and genotypic characterization studies.

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