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Pathogenic Bacteria Associated with Lucilia Sericata from Decomposing Carcasses: Forensic and Public Health Implications

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Key words:
Forensic entomology; Lucilia sericata; Pathogenic bacteria; Public health; Postmortem microbiome

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ABSTRACT

Background: *Blowflies, especially Lucilia sericata, are key to forensic entomology and may act as vectors of pathogenic bacteria. Exploring their microbial associations enhances understanding of both forensic and public health contexts.* ***Objective:*** *This study examined bacterial communities from a decomposing rabbit carcass and adult L. sericata to assess their forensic and epidemiological significance.* ***Methodology:*** *Carcass and fly samples were analyzed using selective culturing, biochemical tests, DNA barcoding, and VITEK-2 profiling. Diversity and similarity were evaluated using Shannon, Simpson, and Bray–Curtis indices.* ***Results:*** *Sixty-five bacterial isolates were identified (32 from the carcass, 33 from flies). Bacillus spp. predominated, alongside clinically relevant taxa (E. coli, S. typhi, S. dysenteriae, S. aureus, S. warneri, P. aeruginosa). Diversity was higher in the carcass (H′ = 1.42; 1–D = 0.70) than in flies (H′ = 1.09; 1–D = 0.58), with a Bray–Curtis similarity of 0.74 indicating strong overlap.* ***Conclusion:*** *Blowflies both mirror and disseminate postmortem bacterial communities, confirming their dual role as forensic indicators and public health vectors. Despite being based on a single carcass, the study underscores the importance of integrating microbial and entomological data in forensic and epidemiological investigations.*

INTRODUCTION

In recent years, the convergence of forensic entomology and forensic microbiology has provided complementary insights into postmortem processes. Necrophagous blowflies (*Diptera: Calliphoridae*), particularly *Lucilia sericata* (Meigen, 1826), are among the earliest colonizers of decomposing remains and serve as key indicators for postmortem interval (PMI) estimation through their developmental patterns. This species is globally recognized for its forensic, medical, and veterinary relevance [1,2].

Microbial communities associated with decomposing remains, collectively termed the thanatomicrobiome, have also proven valuable for estimating decomposition stage and PMI. These communities undergo structured shifts influenced by intrinsic host factors and environmental conditions. At the insect–microbe interface, blowflies acquire, retain, and disseminate bacterial taxa, functioning both as "microbial mirrors" reflecting their larval substrates and as mechanical vectors transmitting pathogenic and antibiotic-resistant bacteria[3, 4].

L. sericata is known to harbor medically important bacteria such as *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, along with other taxa of forensic relevance including *Bacillus* spp., *Salmonella typhi*, and *Shigella dysenteriae*. These bacteria can be transmitted through surface contact, regurgitation, or fecal deposition, and some taxa may persist across generations, suggesting transgenerational transmission and underscoring the epidemiological importance of blowflies in pathogen dissemination [5, 6].

Despite advances in culture-independent and sequencing approaches, integrated studies linking microbial and insect succession remain limited, particularly in the Middle East and North Africa, where unique climatic conditions may influence decomposition dynamics [7]. The present study therefore aims to characterize and compare the culturable bacterial communities of decomposing rabbit (*Oryctolagus cuniculus*) carcass and adult *L. sericata*, evaluating diversity, overlap (as measured by the Bray–Curtis similarity coefficient), and their combined forensic and public health implications. This model was chosen as a decomposition model because small mammalian carcasses are widely used in forensic entomology owing to their manageable size, ethical accessibility, and decomposition patterns comparable to larger mammals[8].

METHODOLOGY

Carcass preparation

A rabbit (*Oryctolagus cuniculus*), weighing about 1.5 kg, was humanely euthanized using CO_2 inhalation in accordance with the AVMA Guidelines for the

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Euthanasia of Animals (2020)[9]. The specimen was acquired on September 20 from the Bani Haritha neighborhood in Al Madinah Al Munawwarah, Kingdom of Saudi Arabia (24°29'28.6"N, 39°38'01.7"E).

Environmental conditions

At the time of carcass placement, the ambient temperature and relative humidity were recorded as 31 °C and 54%, respectively. To facilitate decomposition, the carcass was left in a wire trap that was secured and exposed to the elements [8], allowing natural access of insects, air, and sunlight while preventing interference from scavenging animals.

Sampling procedure

Seven days postmortem, the rabbit carcass was swabbed at 9:00 a.m. using sterile cotton swabs from the external surface of the abdominal region. Each swab was immediately transferred into a sterile container and stored at 4 °C until further microbiological processing[10].

Insect collection

Adult blowflies (*Lucilia sericata*) visiting the cadaver during decomposition were collected using a standard hand net. Captured specimens were transferred into sterilized Falcon polypropylene tubes, which were maintained at 4 °C until identification and subsequent laboratory analysis [11].

DNA barcoding for identification of *Lucilia sericata*

DNA extraction

Total genomic DNA was extracted from individual adult flies using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol with minor modifications to improve extraction efficiency from insect tissues. Specifically, samples were incubated with proteinase K for 3 h at 56 °C instead of 1–2 h, and the elution step was performed twice with 50 µL of AE buffer preheated to 70 °C to maximize DNA yield. To preserve voucher specimens, only a leg or thoracic muscle was used per sample. DNA quality and concentration were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA), with A260/A280 ratios ranging from 1.7 to 1.9, indicating acceptable DNA purity and confirmed by 1% agarose gel electrophoresis [12].

PCR amplification

The mitochondrial cytochrome oxidase I (COI) gene, a standard DNA barcode for dipteran species [13], was amplified in a 25 µL reaction containing 12.5 µL of 2× PCR Master Mix (Thermo Scientific), 1 µL of each primer (10 µM), 2 µL of DNA (~50 ng), and nuclease-free water. Universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAATCA-3′) were used. PCR cycling conditions were: initial denaturation at 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 50 °C for 45 s, and 72 °C for 1 min; followed by a final extension at 72 °C for 10 min [14].

PCR product Aanalysis and sequencing

PCR products were separated on 1.5% agarose gels stained with ethidium bromide and visualized under UV illumination. Purified amplicons (~658 bp) obtained using the QIAquick PCR Purification Kit (Qiagen, Germany) were subjected to bidirectional Sanger sequencing (Macrogen Inc., Korea)

Molecular characterization and phylogenetic analysis

Raw chromatograms were edited and verified using BioEdit v7.2.5, and consensus sequences were aligned with related GenBank entries using ClustalW in MEGA v11 [15]. Species identification was confirmed through BLASTn analysis against the NCBI database. Phylogenetic relationships were inferred using the Maximum Likelihood method with the best-fit substitution model determined by ModelFinder in IQ-TREE v2, with node support evaluated by SH-aLRT tests and 1000 ultrafast bootstrap replicates [16].

Bacterial isolation and heat treatment procedure

Each carcass swab and whole adult fly specimen was aseptically placed in 10 mL of saline solution containing 0.1% Tween 80 and incubated aerobically at 37 °C with shaking (150 rpm) for 4 h to promote bacterial release. Serial tenfold dilutions (10^{-1}–10^{-4}) were then prepared using sterile physiological saline (0.85% NaCl) and 100 µL aliquots were spread on various culture media for bacterial isolation. For *Bacillus* spp. selection, 1 mL of the 10^{-4} dilution was heat-treated at 80 °C for 10 min, cooled, and plated onto Plate Count Agar (PCA) supplemented with 10 µg/mL Polymyxin B sulfate (HiMedia, USA), following the method described by Ranjan et al. (2022)[17].

Enrichment, selective media, and preliminary characterization of bacteria

Various enrichment and selective media were used for bacterial isolation and enumeration, including Plate Count Agar (PCA; Merck, Germany) for total viable counts, Eosin Methylene Blue Agar (Condalab, Spain) for Enterobacteriaceae, Cetrimide Agar (Merck, Germany) for *Pseudomonas* spp., MacConkey Agar (Biolife, Italy) for Gram-negative enterics, *Salmonella–Shigella* Agar (NEOGEN, Canada) for enteric pathogens, and Mannitol Salt Agar (Biolife, Italy) for *Staphylococcus* spp. All inoculated plates were incubated aerobically at 37 °C for 24–48 h to allow optimal bacterial growth and colony development.

Preliminary identification was based on colony morphology, Gram staining, catalase reaction, and the 3% KOH string test to differentiate Gram-positive from Gram-negative bacteria , with all tests performed at room temperature (25 ± 2 °C) [18,19].

Automated identification

Representative isolates (one per bacterial species per source: carcass or fly), totaling 10 isolates, were subjected to confirmatory biochemical identification using the VITEK-2 Compact System (bioMérieux,

France). Identification was performed using GN, GP, and BCL identification cards according to the Gram reaction and microscopic morphology of the isolates, and results were accepted when the probability score was ≥95% [20].

Diversity and similarity analysis

Bacterial community data obtained from both the rabbit carcass and *Lucilia sericata* isolates were analyzed to evaluate within- and between-source diversity. The Shannon diversity index (H′) was calculated for each source to quantify species richness and evenness. The index was computed as:

$$H' = -\Sigma\, p_i \ln(p_i) \quad (1)$$

-Where pi represents the relative abundance of taxon i.

To assess the similarity in bacterial composition between the carcass and flies, the Bray–Curtis similarity coefficient was computed using isolate abundance tables. The metric was expressed as:

$$BC = 1 - \sum|x_i - y_i| / \sum|x_i + y_i| \quad (2)$$

-Where xi and Yi denote the abundances of taxon i in the carcass and fly communities, respectively.

-Values of BC range from 0 (completely dissimilar) to 1 (identical) [21].

Statistical analysis

All statistical analyses and diversity metrics were conducted using PAST v4.12 [22] and SPSS v25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (mean ± SD) were calculated where appropriate. Differences between carcass and fly bacterial communities were assessed using the Shannon diversity index (H′) and Bray–Curtis similarity coefficient (BC), with statistical significance set at $p < 0.05$.

Ethical approval statement

All animal experiments were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. The experimental procedures involving rabbits were performed following ethical standards and approved protocols of the Department of Zoology, Faculty of science, Al-Azhar University, under approval number [AZU-ZOO-2024-01].

RESULTS

Genetic identification of *Lucilia sericata*

PCR amplification of the mitochondrial cytochrome oxidase subunit I (COI) gene from adult *L. sericata* yielded a ~ 610 bp fragment. The obtained sequence (GenBank accession no. PX394753) showed 99.33% similarity to a Korean reference strain (*L. sericata*, EU880209.1). Phylogenetic analysis based on the COI region (**Figure 1**) placed all *L. sericata* sequences in a strongly supported monophyletic clade (bootstrap ≥70%), clearly separated from the outgroup *Wohlfahrtia magnifica*, confirming species-level resolution.

Bacterial isolation and preliminary characterization

A total of 65 bacterial isolates were obtained; 32 from the decomposing rabbit carcass and 33 from *Lucilia sericata* flies (**Tables 1–4**). Quantitative analysis showed bacterial loads of 161 × 10^4 CFU/mL in the carcass and 185 × 10^4 CFU/mL in flies. Preliminary tests (Gram staining, catalase, and KOH assays) differentiated isolates into Gram-positive and Gram-negative groups (**Figure 2**). Both sources were dominated by *Bacillus* spp., alongside clinically important taxa such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (**Table 3**). A comparative heatmap (**Figure 3**) confirmed the predominance and resilience of *Bacillus* spp. across samples.

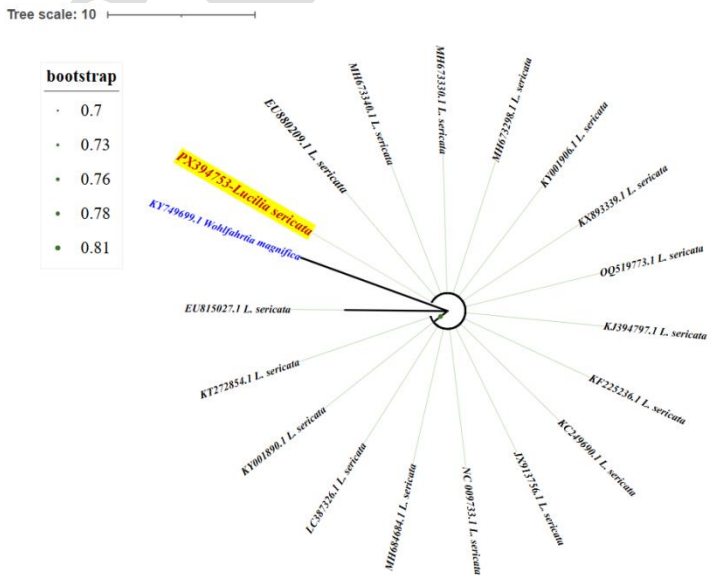

Fig. 1: Phylogenetic tree of *Lucilia sericata* based on mitochondrial COI gene sequences (with bootstrap values ≥70% indicated; PX394753 highlighted as the focal sample; *Wohlfahrtia magnifica* used as outgroup).

Table 1: Growth Characteristics and Preliminary Identification of Bacteria isolated from asphyxia-killed rabbit cadaver on selective media.

No	Code	EMB	CA	MC	SS	MS	PCAB	Gram stain	Catalase	KOH
1	RS1	-	-	-	-	-	+	+	+	-
2	RS2	+/GMS	-	+/RV	-	-	-	-	+	+
3	RS3	-	-	-	-	-	+	+	+	-
4	RS4	- /C	-	+/C	+/C, BC	-	-	-	+	+
5	RS5	+/GMS	-	+/RV	-	-	-	-	+	+
6	RS6	-	-	-	-	+	-	+	+	-
7	RS7	+/GMS	-	+/RV	-	-	-	-	+	+
8	RS8	-	-	-	-	-	+	+	+	-
9	RS9	-	-	-	-	-	+	+	+	-
10	RS10	- /C	-	+/C	+/C, BC	-	-	-	+	+
11	RS11	+/GMS	-	+/RV	-	-	-	-	+	+
12	RS12	-	-	-	-	-	+	+	+	-
13	RS13	+/GMS	-	+/RV	-	-	-	-	+	+
14	RS14	-	-	-	-	-	+	+	+	-
15	RS15	+/GMS	-	+/RV	-	-	-	-	+	+
16	RS16	-	-	-	-	-	+	+	+	-
17	RS17	-	-	-	-	+	-	+	+	-
18	RS18	-	-	-	-	-	+	+	+	-
19	RS19	- /C	-	+/C	+/C, BC	-	-	-	+	+
20	RS20	-	-	-	-	-	+	+	+	-
21	RS21	+	-	+	+	-	-	-	+	+
22	RS22	-	-	-	-	-	+	+	+	-
23	RS23	- /C	-	+/C	+/C, BC	-	-	-	+	+
24	RS24	+/C	+	+	-	-	-	+	+	-
25	RS25	-	-	-	-	-	+	+	+	-
26	RS26	-	-	-	-	+	-	+	+	-
27	RS27	+/GMS	-	+/RV	-	-	-	-	+	+
28	RS28	-	-	-	-	-	+	+	+	-
29	RS29	-	-	-	-	-	+	+	+	-
30	RS30	- /C	-	+/C	+/C, BC	-	-	-	+	+
31	RS31	-	-	-	-	-	+	+	+	-
32	RS32	-	-	-	-	-	+	+	+	-

EMB= Eosin Methylene Blue Agar, CA= Cetrimide Agar, MC= MacConkey Agar, SS= Salmonella-Shigella Agar, MS= Mannitol Salt Agar, PCAB= Plate Count Agar + 10 µg/mL Polymyxin B sulfate, C= colorless, GMS= green metallic shine, BC= black center, and RV= red violet.

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Table 2: preliminary identification of Bacteria isolated from asphyxia-killed rabbit cadaver.

No	Code	Preliminary identification
1	RS1	*Bacillus* sp
2	RS2	*Escherichia coli*
3	RS3	*Bacillus* sp
4	RS4	*Salmonella* sp
5	RS5	*Escherichia coli*
6	RS6	*Staphylococcus aureus*
7	RS7	*Escherichia coli*
8	RS8	*Bacillus* sp
9	RS9	*Bacillus* sp
10	RS10	*Salmonella* sp
11	RS11	*Escherichia coli*
12	RS12	*Bacillus* sp
13	RS13	*Escherichia coli*
14	RS14	*Bacillus* sp
15	RS15	*Escherichia coli*
16	RS16	*Bacillus* sp
17	RS17	*Staphylococcus aureus*
18	RS18	*Bacillus* sp
19	RS19	*Salmonella* sp
20	RS20	*Bacillus* sp
21	RS21	*Shigella* sp
22	RS22	*Bacillus* sp
23	RS23	*Salmonella* sp
24	RS24	*Pseudomonas aeruginosa*
25	RS25	*Bacillus* sp
26	RS26	*Staphylococcus aureus*
27	RS27	*Escherichia coli*
28	RS28	*Bacillus* sp
29	RS29	*Bacillus* sp
30	RS30	*Salmonella* sp
31	RS31	*Bacillus* sp
32	RS32	*Bacillus* sp

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Table 3: Growth Characteristics and Preliminary Identification of Bacteria from *Lucilia sericata* Flies Associated with an Asphyxiated Rabbit Cadaver (Rab/A).

No	Code	EMB	CA	MC	SS	MS	PCAB	Gram stain	Catalase	KOH
1	RL1	-	-	-	-	-	+	+	+	-
2	RL2	-	-	-	-	-	+	+	+	-
3	RL3	+/GMS	-	+/RV	-	-	-	-	+	+
4	RL4	-	-	-	-	-	+	+	+	-
5	RL5	-	-	-	-	+/W	-	+	+	-
6	RL6	-	-	-	-	-	+	+	+	-
7	RL7	-	-	-	-	-	+	+	+	-
8	RL8	- /C	-	+/C	+/C, BC	-	-	-	+	+
9	RL9	-	-	-	-	-	+	+	+	-
10	RL10	+/GMS	-	+/RV	-	-	-	-	+	+
11	RL11	-	-	-	-	-	+	+	+	-
12	RL12	-	-	-	-	-	+	+	+	-
13	RL13	-	-	-	-	+/W	-	+	+	-
14	RL14	-	-	-	-	-	+	+	+	-
16	RL15	-	-	-	-	-	+	+	+	-
16	RL16	-	-	-	-	+/W	-	+	+	-
17	RL17	-	-	-	-	-	+	+	+	-
18	RL18	-	-	-	-	-	+	+	+	-
19	RL19	-	-	-	-	-	+	+	+	-
20	RL20	+/GMS	-	+/RV	-	-	-	-	+	+
21	RL21	-	-	-	-	-	+	+	+	-
22	RL22	-	-	-	-	-	+	+	+	-
23	RL23	-	-	-	-	-	+	+	+	-
24	RL24	- /C	-	+/C	+/C, BC	-	-	-	+	+
25	RL25	-	-	-	-	-	+	+	+	-
26	RL26	-	-	-	-	+/W	-	+	+	-
27	RL27	-	-	-	-	-	+	+	+	-
28	RL28	+/GMS	-	+/RV	-	-	-	-	+	+
29	RL29	-	-	-	-	-	+	+	+	-
30	RL30	+/GMS	-	+/RV	-	-	-	-	+	+
31	RL31	-	-	-	-	-	+	+	+	-
32	RL32	- /C	-	+/C	+/C, BC	-	-	-	+	+
33	RL33	+/GMS	-	+/RV	-	-	-	-	+	+

EMB= Eosin Methylene Blue Agar, CA= Cetrimide Agar, MC= MacConkey Agar, SS= Salmonella-Shigella Agar, MS= Mannitol Salt Agar, PCAB= Plate Count Agar + 10 µg/mL Polymyxin B sulfate, C= colorless, GMS= green metallic shine, BC= black center, W= weak growth, and RV= red violet.

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Table 4: preliminary identification of Bacteria isolated from *Lucillia* sp Flies Associated with an Asphyxiated Rabbit Cadaver (Rab/A).

No	Code	Preliminary identification
1	RL1	*Bacillus* sp
2	RL2	*Bacillus* sp
3	RL3	*Escherichia coli*
4	RL4	*Bacillus* sp
5	RL5	*Staphylococcus* sp
6	RL6	*Bacillus* sp
7	RL7	*Bacillus* sp
8	RL8	*Salmonella* sp
9	RL9	*Bacillus* sp
10	RL10	*Escherichia coli*
11	RL11	*Bacillus* sp
12	RL12	*Bacillus* sp
13	RL13	*Staphylococcus* sp
14	RL14	*Bacillus* sp
15	RL15	*Bacillus* sp
16	RL16	*Staphylococcus* sp
17	RL17	*Bacillus* sp
18	RL18	*Bacillus* sp
19	RL19	*Bacillus* sp
20	RL20	*Escherichia coli*
21	RL21	*Bacillus* sp
22	RL22	*Bacillus* sp
23	RL23	*Bacillus* sp
24	RL24	*Salmonella* sp
25	RL25	*Bacillus* sp
26	RL26	*Staphylococcus* sp
27	RL27	*Bacillus* sp
28	RL28	*Escherichia coli*
29	RL29	*Bacillus* sp
30	RL30	*Escherichia coli*
31	RL31	*Bacillus* sp
32	RL32	*Salmonella* sp
33	RL33	*Escherichia coli*

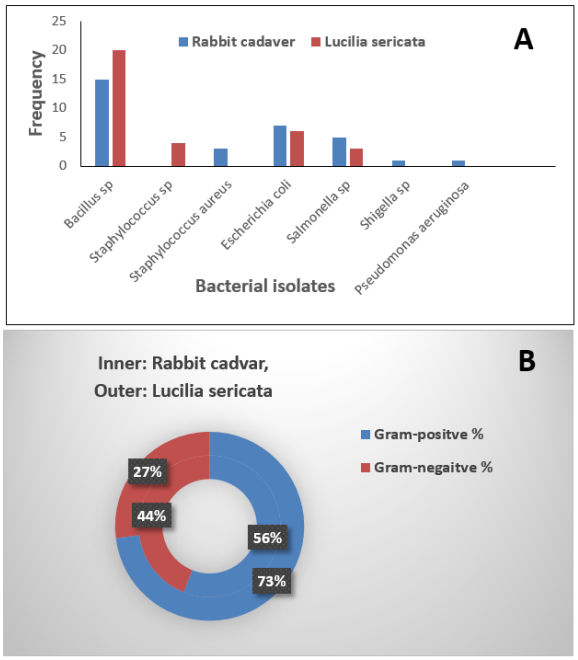

Fig. 2: Comparative visualization of bacterial isolates recovered from the rabbit cadaver and *Lucilia sericata*. (A) Bar chart showing the frequency of individual bacterial taxa in both sources. (B) Proportional distribution of Gram-positive and Gram-negative isolates, with the inner ring representing the rabbit cadaver and the outer ring representing *L. sericata*.

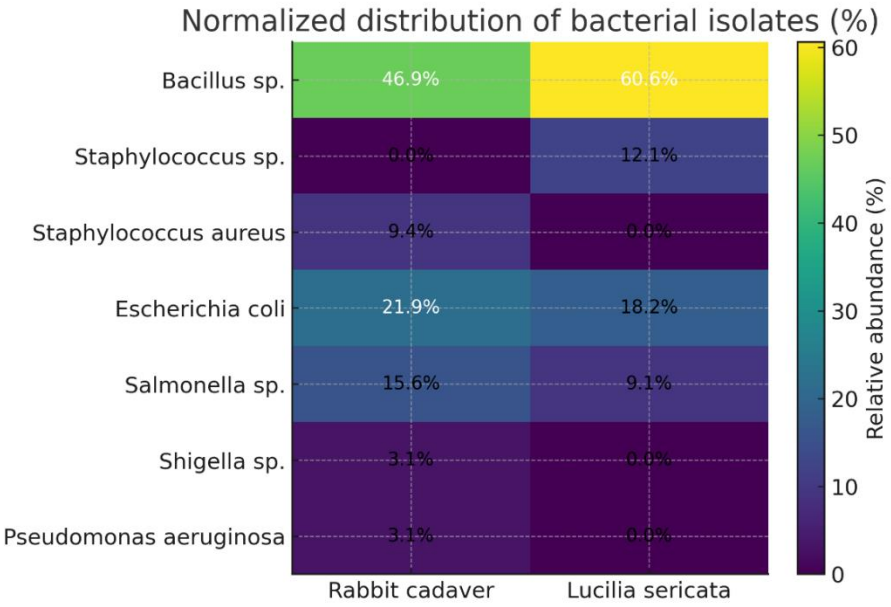

Fig. 3: Heatmap visualization of bacterial isolates recovered from the rabbit cadaver and *Lucilia sericata*, showing both absolute and normalized relative abundances.

Automated identification of bacterial isolates

One representative isolate per bacterial species from each source (*L. sericata* or carcass) was analyzed using the VITEK-2 Compact System to minimize redundancy. Automated profiling confirmed a diverse assemblage of clinically relevant taxa. From the carcass, six species were identified: *Bacillus thuringiensis*, *Escherichia coli*, *Salmonella typhi, Staphylococcus aureus*, *Shigella dysenteriae*, and *Pseudomonas aeruginosa* (≥97% confidence). From flies, four species were detected: *Bacillus subtilis*, *Escherichia. coli*, *Salmonella typhi*, and *Staphylococcus warneri* (**Table 5, Figure 4**).

Diversity indices indicated higher richness in the carcass (H′ = 1.42; 1–D = 0.70) than in flies (H′ = 1.09; 1–D = 0.58). Bray–Curtis similarity (0.74) revealed substantial community overlap, with *E. coli* and *S. typhi* shared between both sources, while *S. aureus* and *Shigella dysenteriae* were exclusive to the carcass, and *S. warneri* to the flies. Rarefaction curves (**Figure 5**) plateaued around 30 isolates, confirming adequate sampling coverage.

Table 5: Bacterial Identification of Isolates from Rabbit Carcass and *Lucilia sericata* using the VITEK-2 System

Source	Isolate code	Preliminary ID	Confirmed ID (VITEK-2)	Probability (%)
Rabbit carcass	RS1	*Bacillus* sp	*Bacillus thuringiensis*	97
	RS2	*Escherichia coli*	*Escherichia coli*	99
	RS4	*Salmonella* sp	*Salmonella typhi*	99
	RS6	*Staphylococcus aureus*	*Staphylococcus aureus*	99
	RS21	*Shigella* sp	*Shigella dysentery*	99
	RS24	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	99
Lucilia sericata	RL1	*Bacillus* sp	*Bacillus subtilis*	97
	RL3	*Escherichia coli*	*Escherichia coli*	99
	RL5	*Staphylococcus* sp	*Staphylococcus warneri*	97
	RL8	*Salmonella* sp	*Salmonella typhi*	99

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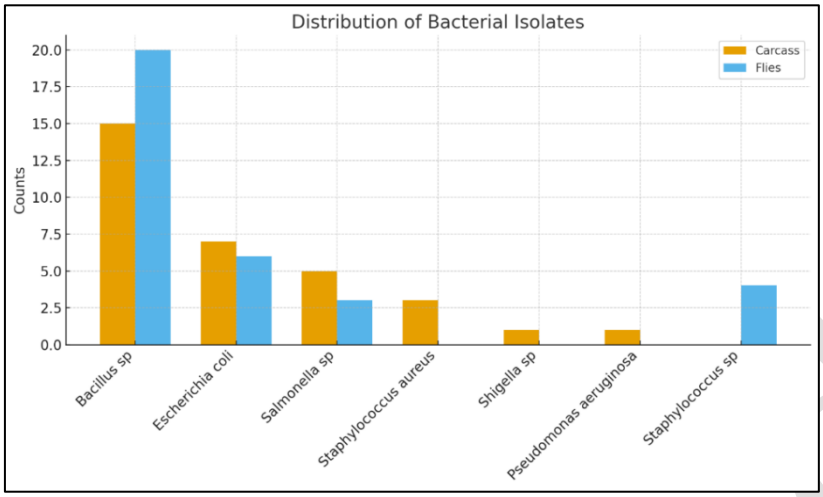

Fig. 4: Distribution of bacterial isolates recovered from the rabbit carcass and *Lucilia sericata* flies.

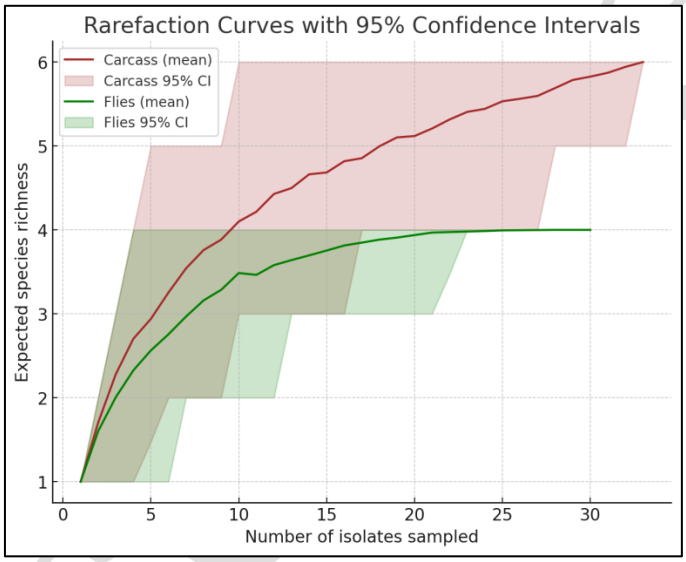

Fig. 5: Rarefaction curves with 95% confidence intervals for bacterial isolates from the rabbit carcass (brown) and *Lucilia sericata* adults (green), showing sufficient sampling to capture dominant taxa.

DISCUSSION

The present study provides new insights into the bacterial communities associated with *Lucilia sericata* and decomposing rabbit carcasses, highlighting their forensic and public health relevance. The findings are discussed below in the context of molecular identification, microbial diversity, and ecological interactions.

Reliability of COI barcoding for forensic blowfly identification

The findings confirm the reliability of the COI gene as a molecular marker for distinguishing forensically important blowflies [23]. Strong bootstrap support aligns with previous evidence of low intraspecific and high interspecific variation within Calliphoridae. The inclusion of *Wohlfahrtia magnifica* as an outgroup strengthened tree topology, consistent with phylogenetic best practices [24]. High sequence similarity across distant populations indicates strong genetic conservation in *L. sericata*, supporting the broad applicability of COI barcoding in forensic and medical entomology for accurate and rapid species identification [25, 26]. Collectively, these results provide robust molecular confirmation of *L. sericata* identity, reinforcing its value as a dependable forensic indicator

Significance of culture-based approaches and pathogen recovery

The recovery of 65 bacterial isolates from carcass tissues and *L. sericata* confirms the efficiency of culture-based methods as a primary tool in forensic microbiology. Selective and differential media (e.g., EMB, MacConkey, SS, MSA, and PCA with Polymyxin B) enabled targeted isolation of enteric pathogens, Gram-positive cocci, and spore-forming *Bacillus* spp.,

supporting previous findings on the reliability of conventional techniques in complex samples [27, 28].

The slightly higher bacterial load in flies indicates that *L. sericata* not only reflects but also amplifies microbial communities, reinforcing its dual role as a microbial mirror and mechanical vector. The dominance of *Bacillus* spp. reflects their spore-forming resilience, while the detection of pathogens such as *E. coli*, *Salmonella*, *Shigella*, *S. aureus*, and *P. aeruginosa* underscores the public health relevance of blowfly activity [29–32]. Overall, these results highlight the importance of integrating traditional culture-based and molecular approaches for forensic and epidemiological investigations, especially in resource-limited contexts

Overlap and divergence between carcass and fly-associated bacteria

Automated identification revealed both shared and unique bacterial taxa between carcass and fly samples, emphasizing the dual function of blowflies as microbial mirrors and selective vectors. The presence of *E. coli* and *S. typhi* in both sources supports the established role of necrophagous flies as carriers of enteric pathogens [32]. Conversely, the restriction of *S. aureus* and *Shigella dysenteriae* to the carcass suggests ecological filtering within the insect gut or cuticle, limiting the transfer of certain taxa [31].

Resilience of *Bacillus* and continuum of *Staphylococcus*

The detection of *Bacillus thuringiensis* and *B. subtilis* across both carcass and flies reflects the ecological resilience of spore-forming taxa, which persist under decomposition-associated stresses [33-36]. Similarly, the concurrent detection of *S. aureus* (carcass) and *S. warneri* (flies) illustrates a continuum between virulent and opportunistic *staphylococci*. Coagulase-negative *staphylococci* (CoNS), long regarded as commensals, are now recognized as important opportunists capable of biofilm formation and harboring resistance genes [37, 38].

Community diversity and ecological filtering

Diversity indices showed higher richness and evenness in carcass communities than in flies, suggesting that flies represent a filtered subset of the thanatomicrobiome. This is consistent with selective retention: while some pathogens thrive in fly-associated microhabitats (e.g. *S. warneri*), others such as *S. aureus* may be poorly adapted to insect environments [39, 40].

Forensic and public health significance

From a forensic standpoint, the findings indicate that blowflies do not uniformly transmit all carcass-associated bacteria; instead, their microbiota reflects selective acquisition and persistence, underscoring their utility in microbial succession studies while cautioning against overgeneralization [41]. The persistence of *E. coli* and *Salmonella* within fly guts is likely supported by biofilm formation and stress tolerance, facilitating bacterial dissemination [42]. Moreover, blowflies are increasingly recognized as carriers of antimicrobial resistance genes (ARGs), including *bla*, *mecA*, and *qnr* determinants [43]. Future studies should incorporate ARG screening to connect forensic entomology with antimicrobial resistance surveillance. Integrating thanatomicrobiome data with insect-borne bacteria could enhance postmortem interval (PMI) estimation and position blowflies as sentinels for pathogen and ARG monitoring in both forensic and public health contexts [34].

Limitations and future perspectives

Although this study provides valuable baseline data, it was limited to a single carcass model under specific environmental conditions. Expanding sampling across different seasons, carcass types, and geographical regions, coupled with metagenomic sequencing, would provide a more comprehensive understanding of microbial-insect interactions in forensic contexts.

CONCLUSIONS

This study demonstrates that *Lucilia sericata* associated with decomposing rabbit carcasses can harbor and transmit pathogenic bacteria of both forensic and public health significance. The detection of shared isolates such as *Escherichia coli* and *Salmonella typhi* underscores the role of blowflies as mechanical carriers and potential forensic indicators, whereas unique taxa highlight selective bacterial acquisition and associated epidemiological risks. These findings support the integration of microbiological and entomological evidence in forensic investigations. Further molecular and metagenomic studies are recommended to elucidate bacterial transfer dynamics and enhance the forensic value of insect-associated microbiota.

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