



Identification of Bacterial Species Isolated from the Legs of *Chrysomya* Screw Flies Using the VITEK 2 System

Shifaa Waleed Khaled ¹, Ammar Abdulwahid Jbarah Al-Karawi ², Aseel Jawad Kadim ¹, Luay Qasim Abdulhameed ¹

¹ University of Diyala, College of Education for Pure Sciences, Department of Biology, Diyala, 32001, Iraq

² The General Directorate for Education of Diyala, 32001 Baqubah, Diyala, 32001, Iraq

¹shifaabio2121@uodiyala.edu.iq, ²ammar.alkraw991@gmail.com, ³bioe.aseel.jwad@uodiyala.edu.iq,

⁴loai.qassim@uodiyala.edu.iq

Article history:

Received: 17 March 2026

Accepted: 18 May 2026

Published: 15 Jun 2026

Keywords: *Chrysomya*, *Chrysomya megacephala*, *Chrysomya albiceps*, *Chrysomya putoria*, *Proteus spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, VITEK 2

This article is open-access under the CC BY 4.0 license (<http://creativecommons.org/licenses/by/4.0/>).

Abstract

This study deals with the identification and diagnosis of bacteria carried on the legs of screw flies of the genus *Chrysomya* using the VITEK 2 technique, due to its medical and veterinary importance as a mechanical vector for pathogens. The study was conducted in the field in agricultural areas (orchards and barns) in Diyala Governorate. Samples were collected from three species: *Chrysomya megacephala*, *C. albiceps*, and *C. putoria*. Fermented food traps, which have proven highly effective in attracting flies, were used. A total of 300 flies were collected, with 100 flies from each species. Bacteria were isolated from the flies' legs after dissection and suspension of the samples in saline solution, and then cultured on different culture media, followed by identification using the VITEK 2 system, which is based on precise biochemical analysis. The results show the presence of three main groups of bacteria: *Proteus spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Out of 104 bacterial isolates, *Proteus spp.* had the highest prevalence (43.27%), followed by *Pseudomonas aeruginosa* (32.69%) and finally *Staphylococcus aureus* (24.04%). Regarding their capacity to act as bacterial reservoirs, *C. megacephala* had the highest capacity compared with other species studied; no statistically significant differences emerged between the various fly species concerning their ability to transmit bacteria. Therefore, the study provides confirmation that screw-flies can be potent vectors for pathogenic bacteria, especially in agricultural settings, thus making them important contributors to disease transmission and indicating the need for monitoring these insects in order to reduce public health hazards.

1. Introduction

Screw flies of the genus *Chrysomya* are insects of medical and veterinary importance, due to their potential role in transmitting pathogens among the environment, animals, and humans [1]. This genus belongs to the family Calliphoridae and is characterized by a widespread distribution in both urban and rural environments, as well as its association with organic waste and carrion, making these flies suitable carriers and reservoirs for many microorganisms [2]. Furthermore, the diversity of insects in agricultural environments, including Diyala Governorate, Iraq, underscores the importance of studying their taxonomy and ecological role. Numerous insect species of ecological and medical importance have been reported in

the region [3]. Numerous studies indicate that *Chrysomya* species, particularly *Chrysomya megacephala*, play an important role as mechanical vectors of various pathogenic bacteria, as they can carry these microorganisms on their body surfaces, especially their legs and mouthparts, as a result of direct contact with contaminated sources such as feces and carrion [4]. Various bacterial species have also been isolated from these insects, including species of medical and veterinary importance, which underscores their role in disease transmission [5].

There has been increasing emphasis placed on the study of microorganisms (specifically bacteria) that are present on or in association with various types of flies due to the global concerns regarding the emergence and

spread of antimicrobial resistance. Studies have shown that flies may act as both reservoirs and vectors of resistant bacterial strains. Therefore, identifying the bacterial taxa carried by these insects is an important step to better understand their epidemiological role and the potential health risks associated with those bacteria [6].

In this regard, bacterial diagnosis has improved significantly due to advances in bacterial diagnostic technology, such as the VITEK 2 system, an automated system for analyzing biochemical properties and metabolic patterns of microorganisms, allowing rapid and accurate identification of bacterial species compared to traditional methods [7]. Clinical and research laboratories have widely used this technique to identify both Gram-positive and Gram-negative bacteria using advanced databases [8].

Based on the foregoing, this study focuses on isolating and identifying bacteria associated with the legs of screw-flies in the genus *Chrysomya*, using modern techniques such as VITEK 2. This study contributes to understanding the epidemiological importance of these screw-flies and aids in our understanding of how these insects contribute to pathogen transmission in the environment and how we can help to limit the spread of diseases related to them.

2. Materials and Methods

2.1 Study location

This study was conducted in the Baquba District and Bahraz Region, Diyala Governorate, Iraq, in various environments, including orchards and livestock pens. These areas are characterized by environmental conditions favorable for fly breeding, in terms of the availability of organic matter and high temperatures, which may increase the prevalence of *Chrysomya* species [9].

2.2 Taxonomic Classification of the fly

Species identification was based on established taxonomic keys that rely on morphological characteristics such as body coloration, eye morphology, and wing characteristics [10]. The identified species (Fig. 1) were:

- *Chrysomya albiceps*
- *Chrysomya megacephala*
- *Chrysomya putoria*



Fig. 1 Fly species identified in the present study

2.3 Trap Design and Bait Preparation

Plastic traps (as shown in Fig. 2) were used, consisting of an upper chamber for fly entry and a lower chamber containing the bait. The bait was prepared using the following formula:

- NPK fertilizer: 50 g
- Dry baker's yeast: 5 g (*Saccharomyces cerevisiae*)
- Distilled water: 1 liter

The ingredients were mixed using a magnetic stirrer for 20 minutes to ensure a homogeneous mixture, as the attraction mechanism depends on yeast fermentation, which leads to the release of volatile compounds such as ammonia, alcohol, and organic acid substances known to attract flies [11].



Fig. 2 Trap design and bait preparation

2.4 Sample collection

The traps were set up at various locations within orchards and barns (Fig. 3) and left in the field for 7 days. The flies were then collected and transported to the laboratory in sterile containers. A total of 100 flies from each species (males and females) were selected for testing.



Fig. 3 shows the traps and where to place them in orchards and barns

2.5 Preparation of bacterial samples

The flies were killed by chilling for 2 hours (4°C), and then the legs behind were dissected using sterile instruments. After that, the legs were placed in tubes containing 5 mL of sterile physiological saline (0.85% NaCl). After that, the samples were vortexed for 1–2 minutes to release the bacteria. Legs are considered one of the most important body parts in bacterial transmission due to their direct contact with contaminated surfaces [12].

2.6 Culture Media Preparation

1. Nutrient Agar

The medium was prepared according to the standard composition: Peptone: 5 g, Beef extract: 3 g, NaCl: 5 g Agar: 15 g, Distilled water: 1 L, and pH = 7.0.

2. MacConkey Agar

The medium was prepared according to the standard composition: Peptone: 20 g, Lactose: 10 g, Bile salts: 5 g, NaCl: 5 g, Neutral red: 0.03 g, Agar: 15 g, and Distilled water: 1 L.

3. Blood Agar

It was prepared by adding 5% sterilized sheep blood to Nutrient agar after cooling it to 45–50°C. All media were sterilized using an autoclave at 121°C for 15 minutes [13].

2.7 Bacterial Isolation

The culture media were inoculated using the streaking method, and then the plates were incubated at 37°C for 24–48 hours with 5 replicates per sample.

2.8 Bacterial purification and diagnosis

Various colonies as shown in Fig. 4: (*Proteus*, *Pseudomonas*, *Staphylococcus aureus*) were selected based on their morphological characteristics.



Fig. 4 Identifying the types of bacteria

2.9 Diagnosis using VITEK 2:(fig5)

The VITEK 2 Compact instrument was used as follows: A bacterial suspension (0.5 McFarland) was prepared, and then it was loaded onto VITEK cards; after that, it was inserted into the device for analysis. (*Proteus* spp. and *Pseudomonas aeruginosa*) were identified using GN identification cards, whereas Gram-positive isolates (*Staphylococcus aureus*) were identified using GP identification cards). The device relies on the analysis of

multiple biochemical reactions to identify bacteria with high accuracy [7,8].

2.10 Statistical Analysis

A completely randomized design (CRD) was used, and the results were analyzed and the percentage of bacterial prevalence was calculated. Number of replicates. The chi-square (χ^2) test was used to determine statistically significant differences at a significance level of $p < 0.05$ in the distribution of bacteria among species.

3. Results

4.1 The effectiveness of traps in catching flies

The fermented food traps used in the study proved highly effective in attracting screw flies of the genus *Chrysomya*, with a total of 300 flies collected, 100 flies per species. The identified species included: *Chrysomya megacephala*, *Chrysomya albiceps*, *Chrysomya putoria*. All species were observed at the study sites (orchards and barns in Bahraz/Baquba), with *C. megacephala* being the most abundant species within the traps.

4.2 Isolation and diagnosis of bacteria

Bacterial isolation from fly legs resulted in the growth of multiple colonies on various culture media (nutrient agar, MacConkey agar, blood agar), with these colonies exhibiting differences in morphological characteristics such as color, size, and pattern. The isolates were analyzed using the VITEK 2 system, which detected the following bacterial species: *Proteus* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. It was observed that *Proteus* spp. exhibited characteristic swarming motility on solid media, while *Pseudomonas aeruginosa* was characterized by the production of greenish pigments; *Staphylococcus aureus* colonies, meanwhile, appeared circular and golden in color on blood agar.

4.3 Numerical and proportional distribution of bacterial isolates

A total of 104 bacterial isolates were identified from all samples. These isolates were distributed as follows (Table 1). *Proteus* spp. was the most prevalent, followed by *Pseudomonas aeruginosa*, whereas *Staphylococcus aureus* showed the lowest prevalence.

4.4 Distribution of bacteria by fly species

The results showed differences in the distribution of bacterial isolates among fly species, as shown in the following table (2). The species *C. megacephala* recorded the highest number of bacterial isolates, suggesting that it plays a greater role as a mechanical vector for bacteria compared to other species.

Table 1: The type of bacteria identified, the number of isolates, and their percentage

Bacterial species	Number of isolates	Percentage%
<i>Proteus spp.</i>	45	43.27%
<i>Pseudomonas aeruginosa</i>	34	32.69%
<i>Staphylococcus aureus</i>	25	24.04%
Total	104	100%

Table 2: The distribution of bacterial isolates according to fly species

Fly species	<i>Proteus spp</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	Total
<i>C. megacephala</i>	18	14	10	42
<i>C. albiceps</i>	15	11	8	34
<i>C. putoria</i>	12	9	7	28
Total	45	34	25	104
$\chi^2 = (0.033)$	$df=4$		$p>0.05$	

4. Discussion

The results of the current study indicate that screw flies of the genus *Chrysomya* act as effective mechanical vectors for bacteria, as several medically significant bacterial species were isolated from the flies' legs, most notably *Proteus spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. These findings are consistent with several previous studies that have shown that flies from the family of Calliphoridae act as major vectors of microorganisms owing to the nature of their diet and preference for decaying organic material [14].

The preponderance of *Proteus spp.* in the present study is attributed to the high adaptability of these bacteria and the tendency to thrive in areas rich in organic matter, such as feces and carcasses, areas where flies thrive heavily. Yin et al. (2022) reported that these bacteria are frequently involved in the process of organic matter breakdown, making them more likely to be acquired by vector flies [15]. Similarly, Nazni et al. (2006) reported that house flies and *Chrysomya* species carried similar bacterial species, including species belonging to the genus *Proteus spp.*, which supports the findings of this study [6].

The existence of *Pseudomonas aeruginosa* is associated with its capability to thrive in different environments such as soil, water, and other contaminated surfaces, thus qualifying it as an opportunistic bacterium of significant medical importance [16]. It has been shown in recent scientific investigations that flies act as mechanical vectors for spreading this bacterium, mainly through their legs, which directly touch contaminated surfaces [17].

As far as *Staphylococcus aureus* isolation is concerned, its occurrence represents contamination of the environment of either animal or human nature

because this bacterium is commonly found in animal skin or mucous membranes and easily spread by flies [18]. This shows that flies play an important role in the transmission of pathogens from animals to humans, particularly in mixed farming environments such as barns.

Moreover, the findings revealed that *Chrysomya megacephala* exhibited the greatest number of isolated bacteria in comparison to other fly species. This is attributed to the fact that this species has a higher association with the environment where there is contamination and decomposition of organic materials, thus exposing it to more bacteria [11]. Besides, the behavior of this fly species in feeding and searching for food is more effective when collecting microbes.

Although different fly species carried genetically distinct bacteria, a statistical comparison using the independent chi-square test showed no statistically significant differences among the fly groups. Therefore, all the different types of flies considered in this study have the capacity to carry bacteria. The differences among these fly species may therefore reflect environmental conditions affecting each type of fly differently, rather than any genetically based differences that affect the ability of any type of fly to carry bacteria.

The findings support the theory that screw-flies are more than a mere nuisance and serve as a serious threat to public health through their role as carriers of disease-causing microorganisms within an environmental epidemiological context. This supports the work of Zurek and Ghosh (2014), who state that flies can be used as mobile microbial vectors of contamination between multiple sources [18].

Overall, this study highlights the need for control of flies in agricultural settings, particularly in those agricultural communities where there are interactions between people and livestock that can lead to disease transmission to the human population and the overall public health impact.

5. Conclusion

The findings indicated that flies belonging to the genus *Chrysomya*, found extensively in Bahraz/Baquba-Diyala, are mechanical vectors of bacteria of medical importance since different types of bacteria were found on the legs of these flies. The findings from the isolation and identification process carried out using the VITEK 2 system showed that there were three dominant bacteria, namely *Proteus spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, thus showing the diversity of microorganisms found in these insects. *Proteus spp.* bacteria were present in greater numbers than other species, implying their close relationship with the decaying organic material on which the flies depend for nourishment. It was observed that *C. megacephala* had the greatest number of bacteria, more than the other types tested, proving that there were differences in the effectiveness of mechanical transmission in different species. The chi-square test results did not show any significant difference among fly species concerning the presence of bacteria. This means that all the tested species of flies can effectively transfer bacteria in the same manner. Fermented food traps (using NPK and yeast) have been highly effective in luring flies and thus are a good method to use in the field studies. These findings highlight the significance of screw flies as possible vectors of disease transmission from the environment to animals, especially in areas that harbor both agriculture and livestock, such as barns and orchards.

Conflict of interests

The authors declare no conflict of interest.

Acknowledgments

The authors would like to convey their heartfelt appreciation to the orchard owners, who generously allowed sample collection on their farm and gave invaluable support during the fieldwork. Their collaboration and support considerably aided the successful execution of this investigation.

References

- [1] Khaled, S. A. W., Sultan, A. A., & Atiya, G. A. (2018). A combined technique of GC and mass spectroscopy used for the identification of specific

compounds from *Chrysomya megacephala* and *Chrysomya albiceps* extracts used for the assignments of those species. *Biochemical & Cellular Archives*, 18(2).

- [2] Azevedo, W. T. d. A., Nunes, M. d. P., Machado, T. d. S. T., Albuquerque, V. M. L., Lessa, C. S. S., Alencar, J., & Aguiar, V. M. (2025). Calliphoridae and Mesembrinellidae (Insect: Diptera) Across Different Environments of Rio de Janeiro, Brazil: Synanthropy and Potential Bioindicators, with Notes on Bait Preference. *Life*, 15(12), 1818. <https://doi.org/10.3390/life15121818>.
- [3] Al-Karawi, A. J., & Sultan, A. A. (2022). Molecular Classification for Some Species of Ground Beetles to the Carabidae Family Spread in Diyala Governorate. *Texas Journal of Agriculture and Biological Sciences*, 5, 43-53.
- [4] Kanan, M., Salaki, C., & Mokosuli, Y. S. (2020). Molecular Identification of Bacterial species from *Musca domestica* L. and *Chrysomya megacephala* L. Luwuk City, Central Sulawesi, Indonesia. *J Pure Appl Microbiol*, 14(2), 1595-1607.
- [5] Nair, B. M., & Tomson, M. (2024). Gut microbiome characterisation of *Chrysomya megacephala*: Isolation, identification, antibiotic profiling, and initial documentation of *Leclercia adecarboxylata* from the fly. *Journal of Pure and Applied Microbiology*, 18(4), 2446-2461. <https://doi.org/10.22207/JPAM.18.4.17>.
- [6] Nazni, W. A., Seleena, B., Lee, H. L., Jeffery, J., Rogayah, T. A. R., & Sofian-Azirun, M. (2005). Bacteria fauna from the house fly, *Musca domestica* (L.). *Tropical Biomedicine*, 22(2), 225-231.
- [7] Pincus, D. H. (2006). Microbial identification using the bioMérieux VITEK® 2 system. In *Encyclopedia of rapid microbiological methods* (pp. 1-32). Parenteral Drug Association.
- [8] Becerra, S. C., Roy, D. C., Sanchez, C. J., Christy, R. J., & Burmeister, D. M. (2016). An optimized staining technique for the detection of Gram positive and Gram-negative bacteria within tissue. *BMC research notes*, 9, 216. <https://doi.org/10.1186/s13104-016-1902-0>.
- [9] Ivorra, T., Khorri, S. M., Rahimi, R., & Heo, C. C. (2023). New developmental data of *Chrysomya megacephala* (Diptera: Calliphoridae) in tropical temperatures and its implications in forensic entomology.
- [10] Whitworth, T. (2019). Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America, North of Mexico. In *Forensic entomology* (pp. 413-443). CRC Press.
- [11] Lasa, R., & Williams, T. (2021). Does ammonia released from protein-based attractants modulate

- the capture of *Anastrepha obliqua* (Diptera: Tephritidae)? *Insects*, 12(2), 156. <https://doi.org/10.3390/insects12020156>.
- [12] Sukontason, K. L., Bunchu, N., Methanitikorn, R., Chaiwong, T., Kuntalue, B., & Sukontason, K. (2006). Ultrastructure of adhesive device in fly in families Calliphoridae, Muscidae and Sarcophagidae, and their implication as mechanical carriers of pathogens. *Parasitology Research*, 98(5), 477–481. <https://doi.org/10.1007/s00436-005-0100-0>.
- [13] Atlas, R. M. (2010). *Handbook of microbiological media*. CRC Press.
- [14] Sukontason, K. L., Bunchoo, M., Khantawa, B., Piangjai, S., Rongsriyam, Y., & Sukontason, K. (2007). Comparison between *Musca domestica* and *Chrysomya megacephala* as carriers of bacteria in northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 38(1), 38–44.
- [15] Yin, J.-H., Kelly, P. J., & Wang, C. (2022). Flies as Vectors and Potential Sentinels for Bacterial Pathogens and Antimicrobial Resistance: A Review. *Veterinary Sciences*, 9(6), 300. <https://doi.org/10.3390/vetsci9060300>
- [16] Diggle, S. P., & Whiteley, M. (2020). Microbe Profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. *Microbiology (Reading, England)*, 166(1), 30–33. <https://doi.org/10.1099/mic.0.000860>.
- [17] Hassan, A. O., Uyigue, P. O., Akinleye, A. C., & Oyeromi, O. B. (2021). The role of common housefly as a mechanical vector of pathogenic microorganisms. *Funksec here*, 3(1), 261-269.
- [18] Zurek, L., & Ghosh, A. (2014). Insects represent a link between food animal farms and the urban environment for antibiotic resistance traits. *Applied and environmental microbiology*, 80(12), 3562-3567.