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An Epidemiological of Cutaneous Leishmaniasis in Thi-Qar Province Iraq During 2023–2024

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Abstract

Introduction. Iraq is an area endemic for cutaneous leishmaniasis. It is caused by a protozoan parasite that leaves lifelong scars, but it is not fatal and is considered a neglected disease that spreads under various environmental conditions. Iraq is endemic for cutaneous leishmaniasis. A field study was conducted to shed light on the epidemiology of leishmaniasis in Thi-Qar Province and its districts.
Purpose. The current study aimed to determine the epidemiology of leishmaniasis in Thi-Qar Province and its districts.
Materials and methods. This study included four health centers: Nasiriyah Teaching Hospital, Shatra General Hospital, Rifa'i General Hospital, and Jubayish General Hospital, during the period from the beginning of October 2024 to the end of July 2025. It included different age groups from 1 to 59 years. The study results recorded 95 cases. After clinical diagnosis by a specialist physician, it was confirmed that it was cutaneous leishmaniasis and not another skin disease.
Results. The current study recorded that the infection rate among males was 55.9%, higher than in females 44.21%. Regarding age groups, the highest infection rate was in the under 10 years age group (27.4%). Infection rates reached 28.4% in Nasiriyah district. Based on residential status, the highest infection rate was in urban areas (60%). Regarding the number of lesions, the highest infection rate was 45.3% with a single lesion. As for the location of infection, the highest percentage was 48.1% in the upper extremities. The highest infection rate was recorded in January (37.9%), and the lowest in March (2.1%).
Conclusion. Cutaneous leishmaniasis is a significant health concern in Thi-Qar Province, primarily affecting males, children under ten, and urban populations. Nasiriyah district faces the highest disease burden. Most patients present with a single lesion on their upper extremities. Seasonally, infections peak sharply in January and cease by summer, emphasizing the urgent need for targeted public health disease control strategies.
Keywords: leishmaniasis, leishmania, protozoan, parasite, neglected diseases

■ INTRODUCTION

Leishmaniasis is considered a neglected disease by the World Health Organization, with more than one million cases reported annually [1]. It is a highly variable and

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complex disease in terms of its etiology [2]. There are three types of leishmaniasis: visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis. Each type has its own endemic areas and specific signs and symptoms [3]. The parasite's life cycle occurs within two hosts: a vertebrate host and an invertebrate host. The sand fly is the primary vector for the Leishmania parasite. This insect inhabits tropical regions and reproduces during the summer [4]. During the breeding season, the female feeds on blood, which is essential for egg development. She inserts her proboscis into the bodies of dogs, rodents, and humans, transmitting the Leishmania parasite in its amastigo stage. This stage transforms within her intestines into the infectious flagellated stage [5]. The parasite can then spread to other organs such as the liver and spleen, causing visceral leishmaniasis [6]. Alternatively, it can remain in the skin, causing cutaneous leishmaniasis. Symptoms begin as a lesion at the site of the sand fly bite, which ulcerates and may be accompanied by a secondary bacterial or viral infection [7]. This can leave lifelong scars, and recovery from the disease takes 3–18 months [8]. This disease has several names, such as Baghdad boil, Delhi boil, and Lesser Sister. (WHO 2010). This disease is often associated with a weakened immune system, malnutrition, and poor housing. (WHO 2022) Iraq is one of the countries where leishmaniasis is prevalent. The first case appeared in the capital, Baghdad [9]. Approximately 12,038 cases were recorded among children and rural areas in central and southern Iraq [10]. Recent studies recorded approximately 7,112 cases of infection in Iraq during the years 2011–2013 Al-warid et al. [11]. A study by Hassan [12] confirmed 58 cases of infection in Erbil Province, while stated that infection is more prevalent in the southern regions than in the northern regions. During the period between 2017 and 2018, the number of cases in Thi-Qar Province reached 225 [13]. In 2018 and 2019, the number of cases reached 247 [1, 14], confirmed 125 cases in Thi-Qar Province. The most important factors contributing to the spread of the disease are malnutrition, rural-to-urban migration, socioeconomic conditions, and poor health conditions.

■ MATERIALS AND METHODS

An epidemiological study was conducted in Thi-Qar Province, southern Iraq, from the beginning of October 2023 to the end of July 2024. Ninety-five samples were collected from patients referred to Al-Hussein Teaching Hospital, Al-Shatra General Hospital, Suq Al-Shuyukh General Hospital, and Al-Rifai General Hospital. The patients were of both sexes and ranged in age from 1 to 59 years. A data form was completed, including the following information (name, sex, age, occupation, type of area, location, number of lesions, and presence of animals).

The samples were divided into three groups

1. Group 1: 25 samples that underwent blood tests.
2. Group 2: 25 samples from which skin swabs were taken.
3. Group 3: control group, comprising 10 samples.

Statistical analysis

The data of the current study was statistically analysed by using SPSS version 26, based in using Chi-Square in $p<0.05$ [15, 16].

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■ RESULTS

Distribution of CL infection by Sex

The highest infection rate was among males 53 cases, at a rate of 55.79%, while females were infected with 42 cases, at a rate of 44.21%. The highest percentage of infection was within the age group younger 10–19 years by 27.4%, while the lowest 4.2% was within the age group 50–59. The center of Nasiriyah district had the highest infection rate of 28.4%, while the lowest percentage was 1% in Al-Akikah, Al-Tar and Al-Fadhliah districts. The highest percentage of urban casualties was 57, at a rate of 60%, while the number of injuries in rural areas was38, at a rate of 40% (Table 1).

Table 1
Distribution of CL in relation to the study variables

Sex	**No.**	**%**
Males	53	55.79
Females	42	44.21
p. value 0.230		
Age Groups		
≤10	21	22.11
10–19	26	27.37
20–29	12	12.63
30–39	13	13.68
40–49	19	20.00
50–59	4	4.21
p. value 0.001		
Province Sector		
Nasiriyah	27	28.42
Shatrah	11	11.58
Al Chbayish	2	2.11
Suq Al-Shuyoukh	7	7.37
Al-Rifai	2	2.11
Al-Gharraf	15	15.79
Al-Dawaya	4	4.21
Al-Batha	3	3.16
Al-Qalaa	5	5.26
Al-Islah	2	2.11
Sayed Dakhil	1	1.05
Al-Fadhliah	1	1.05
Al-Tar	1	1.05
Al-Akikah	1	1.05
Garmat Bani Saeed	2	2.11
Al-Nasr	2	2.11
Al-Fajr	2	2.11
Al-Fuhud	4	4.21
Ur	2	2.11
Al-Hmmar	1	1.05
p. value <0.001		
Residence		
Urban	57	60.0
Rural	38	40.0
p. value 0.046		

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The highest percentage of those infected was 51, at a rate of 53.68%, while the lowest percentage was 9, at a rate of 9.47%. The highest percentage of those infected with one lesion was 45.3% with 43 infected, while the lowest percentage was 11.5% for more than three lesions, with 11 infected. The highest percentage 37.9% was recorded in January and the lowest infection rate was in April and May 1%, while July and June did not record any injuries (Table 2).

Table 2
Distribution of CL in relation to the disease variables

Location of Lesion	**No.**	**%**
Upper extremities	51	53.68
Lower extremities	17	17.89
The face	18	18.95
Rest of the body	9	9.47
p. value <0.001		
Number of Lesions		
One lesion	43	45.3
Two lesions	21	22.1
Three lesions	20	21.1
More than three lesions	11	11.5
p. value <0.001		
Month		
October	4	4.21
November	13	13.68
December	18	18.95
January	36	37.89
February	20	21.05
March	2	2.11
April	1	1.05
May	1	1.05
June	0	0.00
July	0	0.00
p. value <0.001		

■ DISCUSSION

The results of the current study showed that the infection rate among males was higher in females. This is attributed to social customs and traditions, where males enjoy greater freedom of movement and play in public places. Males are more exposed to the means of transmission due to these social customs and traditions, in addition to the larger exposed body surface area reflected in their clothing. This study is consistent with most previous studies, including Al-Yasiri [1] study, which found an infection rate of 60.8% among males and 39.2% among females, and OLEIWI et al. [17] study in Thi-Qar Province, which found an infection rate of 66.4% among males and 33.6% among females. It also agrees with Al-Lami [18] study in Maysan Province, which found an infection rate of 52.26% among males and 47.74% among females. These results are consistent with a 2018 study by Younis in Baghdad, where the prevalence was 66.7% among males and 33.3% among females. They also align with a 2014 study by Al-Awadi [13], which found a prevalence of 64.3% among

males and 35.7% among females, as well as a 2016 study by Al-Hassani in Diwaniyah Province, which found a prevalence of 56.4% among males and 53.6% among females. The current study did not agree with Al-Dhafee's 2013 study in Diwaniyah. As for age groups, the highest infection rate was recorded in the under-10 age group, and the lowest in the 50-59 age group. This may be attributed to their lack of awareness of insect bites, in addition to the weaker immune systems of children. Furthermore, adults may have abundant sebum on their faces, which acts as an insect repellent, unlike children who lack this secretion. Also, opening doors and windows and sleeping on rooftops due to frequent power outages, especially in the summer, leads to direct contact between carriers of the disease and people of all ages. The reason for the lower infection rate among the elderly is that their skin is characterized by wrinkles and dryness, making it less attractive to carriers, and because most of them have acquired immunity because of previous infections. The results of this study are consistent with those of Al-Yasiri [1], Al-Taee [19], and OLEIWI et al. [17] in Thi-Qar Province, 18 Al-Lami [18] in Maysan Province.

They also align with Al-Warid et al. [11] in Anbar Province. However, this study does not agree with Musa's (2011) study in Baghdad, where the percentage of those affected ranged between 16 and 40 years old.

The highest rate (28.4%) was recorded in Nasiriyah District, while the lowest rate (1%) was in the districts of Al-Akaika, Al-Tar, Al-Fadhiliya, and Sayyid Dakhil. These areas are considered breeding grounds for insects, and the poor state of municipal services, such as sewage and rainwater overflows and the accumulation of household waste near residential areas, may also be due to the lack of insecticide use and spraying on waste. This study is consistent with the findings of Al-Yasiri [1] and Al-Lami [18] in Maysan Province, Hamzavi et al. [20], and OLEIWI et al. [17] in Thi-Qar Province, and Abul-Doanej [21].

This study showed that the highest infection rate was recorded in January at 37.9%, while no infections were recorded in June and July. This difference may be attributed to the influence of climatic conditions, such as humidity and temperature, on the vector medium. Al-Yasiri [1] study showed that insects begin to appear during August and September, coinciding with increased human exposure. Al-Taee [19] study indicated that the disease appears in winter and spring and disappears during summer. This study consists of the studies by Tarish [22] and Al-Hassani [23] in Al-Diwaniyah Province, OLEIWI et al. [17] study in Thi-Qar Province, and Al-Lami [18] study in Maysan Province, but it is inconsistent with the studies by Seaad et al. [24] in Al-Diwaniyah Province.

The highest infection rate was observed in the upper extremities (48.1%), while the lowest rate was in the rest of the body (12.5%). This is because the infection is concentrated in exposed areas such as the arms, legs, and face, and less so in covered areas such as the back, abdomen, and sides of the body. This study consists of Al-Yasiri [1] study, Al-Awadi [13] study, OLEIWI et al. [17] study in Thi-Qar, Al-Lami [18] study in Maysan, and Younis [25] study in Baghdad.

Regarding the number of lesions, the highest rate (45.3%) was observed with a single lesion, while the lowest rate (11.5%) was observed with more than three lesions. The reason for infection with a single lesion is that the female sandfly typically feeds on only one blood meal. The appearance of more than one lesion indicates that the person has been bitten more than once, either by multiple insect vectors or by rubbing the lesion to spread it to other areas. This study is consistent with Al-Yasiri [1] Al-Khayat et al. [26]. Researcher OLEIWI et al. [17] in Thi-Qar recorded an incidence rate of 55.47% for each

lesion, while it was 23.9% for two lesions. It also aligns with Al-Lami [18] study, where the incidence rate for a single lesion was 53.91%, and the incidence rate for more than three lesions was 11.11%. This is consistent with the results of Daham and Al-Alusi [27] study in Tikrit, Al-Khayat et al. [26] study in Erbil. However, this study is not consistent with Al-Mafraji et al. [28] study in Baghdad, nor with Al-Nassiri and Al-Alousy [29] study in Salah alDin. Regarding the relationship between the disease and type of residence, the highest infection rate was among patients residing in urban areas, numbering 57 (60%), while the lowest infection rate was in rural areas, with 38 patients (40%). This may be attributed to the deterioration of municipal and health services, particularly the accumulation of waste, which serves as a breeding ground for leishmaniasis. Additionally, increased migration from rural to urban areas has led to urban sprawl, with residents settling in agricultural lands. This population density facilitates the transmission of the disease from infected individuals to healthy ones, contributing to the increased spread of the epidemic in cities. This study is consistent with the findings of Al-Lami [18] study in Maysan Province.

■ CONCLUSION

Cutaneous leishmaniasis is a significant health concern in Thi-Qar Province, primarily affecting males, children under ten, and urban populations. Nasiriyah district faces the highest disease burden. Most patients present with a single lesion on their upper extremities. Seasonally, infections peak sharply in January and cease by summer, emphasizing the urgent need for targeted public health disease control strategies.

■ REFERENCES

1. Al-Yasiri F. Immunohistochemistry to identify Leishmania parasites in fixed tissue [MSc thesis]. Thi-Qar: College of Education for Pure Science, University of Thi-Qar; 2022. [Local repository print archive; no public DOI/URL available]
2. Silva H, Liyanage A, Deerasinghe T, et al. Therapeutic response to thermotherapy in cutaneous leishmaniasis treatment failures for sodium stibogluconate: a randomized controlled proof of principle clinical trial. *Am J Trop Med Hyg.* 2021;104(3):945–950.
3. Rashidi S, Mansouri R, Ali-Hassanzadeh M, et al. miRNAs in the regulation of mTOR signaling and host immune responses: the case of Leishmania infections. *Acta Trop.* 2022;232:106431.
4. Ghorbani M, Farhoudi R. Leishmaniasis in humans: drug or vaccine therapy? *Drug Des Devel Ther.* 2018;12:25–40.
5. Sarwar M. Typical flies: Natural history, lifestyle and diversity of Diptera. In: Life Cycle and Development of Diptera. London: IntechOpen; 2020. 1–51 p.
6. Costa CH, Chang KP, Costa DL, Cunha FV. From infection to death: an overview of the pathogenesis of visceral leishmaniasis. *Pathogens.* 2023 Jul 24;12(7):969.
7. Tsai PH, Chen YT, Liau JY, et al. Molecular diagnosis and therapy for cutaneous leishmaniasis of a returned traveler from Mexico. *J Microbiol Immunol Infect*. 2021;54(6):1154–1158.
8. Piscopo TV, Mallia Azzopardi C. Leishmaniasis. *Postgrad Med J.* 2007;83(976):649–657.
9. Taj-Eldin SD, Alousi K. Kala-azar in Iraq: Report of four cases. *J Fac Med Baghdad.* 1954;18(1–2):15–19.
10. Sukker F. Study on sand flies as vectors of Kala-azar in Iraq. *Bull Endem Dis Iraq.* 1974;15:1–2.
11. Al-Warid HS, Al-Saqur IM, Al-Tuwaijari SB, AlZadawi KAM. The distribution of cutaneous leishmaniasis in Iraq: demographic and climate aspects. *Asian Biomed.* 2017;11(3):255–260.
12. Hassan ZI. Molecular characterization of cutaneous leishmaniasis isolated from human in Erbil Province, Kurdistan Region / Iraq. *ZJPAS.* 2018;30(2):76–85.
13. Al-Awadi H. Phenotypic and molecular characterization of sand flies (Diptera: Psychodidae) with molecular and immunological diagnosis of cutaneous leishmaniasis patients in Thi-Qar Province, Iraq. PhD thesis. Thi-Qar: College of Education for Pure Science, University of Thi-Qar; 2019. 194 p.
14. Flaih MH. Epidemiology and molecular study of Leishmania tropica isolated from cutaneous lesions in Thi-Qar Province, Iraq. PhD thesis. Thi-Qar: College of Education for Pure Science, University of Thi-Qar; 2020. 165 p.
15. Ameen IA, Okab HF. Evaluation of immune and inflammatory response in diabetic foot patients and patients presenting diabetes only in Thi-Qar Province / Iraq. *Lab Diagnostics East Eur.* 2024;13(2):52–57.
16. Zubairi RB, Hasan SA, Okab HF. Effect of IL-17 and IL-23 levels in women with polycystic ovary syndrome. *Reprod Heal East Eur.* 2025;15(1):43–50.
17. Oleiwi AH, Al-Khuzaie AA, Mohammed ZJ. Epidemiological study of cutaneous leishmaniasis patients in Thi-Qar Province, Iraq. *Iranian Journal of Ichthyology*. 2021 Mar 5;8:1–7.
18. Al-Lami S. Epidemiological and diagnostic and genetics study for leishmania parasite in Misan government. MSc thesis. Thi-Qar: College of Education for Pure Science, University of Thi-Qar; 2021. 76 p.

19. Al-Taee LH. Study of the Effect of Some Vital Assistance on the Immune Response to Visceral Leishmaniasis in Laboratory Mice. PhD thesis. Kufa: Faculty of Veterinary Medicine, University of Kufa; 2002. 111 p.
20. Hamzavi Y, Khademi N. Trend of cutaneous leishmaniasis in Kermanshah Province, West of Iran from 1990 to 2012. *Iran J Parasitol.* 2015;10(1):78–86.
21. Abul-Doanej HAI. Study of epidemiological aspects for leishmaniasis and diagnosis of the parasite by using nested kinetoplast minicircle DNA-PCR technique in the Province of Maisan-Iraq. MSc thesis. Basrah: College of Education for Pure Science, University of Basrah; 2014. 93 p.
22. Tarish HR. Survey study of cutaneous leishmaniasis in Najaf city. *Kufa Med J.* 2000;3(1):46–49.
23. Al-Hassani MKKT. Epidemiological, molecular and morphological identification of cutaneous leishmaniasis and its insect vectors in Eastern Al-Hamzah district, Al-Qadisiya province. MSc thesis. Al-Qadisiya: College of Education, Al-Qadisiya University; 2016.
24. Seaad AA, Abdulah SR, Kelef SF. Epidemiological prevalence of cutaneous leishmaniasis in immigration people in Al-Qadissyia province. *Al-Anbar J Vet Sci.* 2017;10(1):77–83.
25. Younis NN. Molecular, immunological detection and experimental treatment of cutaneous leishmaniasis in mice and patients (volunteers). PhD thesis. Baghdad: Institute of Genetic Engineering and Biotechnology, University of Baghdad; 2018. 145 p.
26. Al-Khayat ZAY, Agha NFS, Alharmni KIF, Khudhur YJ. A clinico-epidemiological study on cutaneous leishmaniasis in Erbil, Iraq (2015–2017). *Int J Res Dermatol.* 2018;4(1):1–7.
27. Daham AK, Al-Alusi TA. Epidemiological survey of cutaneous leishmaniasis in Salah Al-Din Province. MSc thesis. Tikrit: Department of Biology, College of Education for Girls, Tikrit University; 2011. 601–611 p.
28. Al-Mafraji KH, Al-Rubaey MG, Alkaisy KK. Clinco-epidemiological study of cutaneous leishmaniasis in Al-Yarmouk Teaching Hospital. *Iraqi J Comm Med.* 2008;3:194–197.
29. Al-Nassiri AKD, Al-Alousy TI. Epidemiological survey for cutaneous leishmaniasis in Salah El-Din Province. *Diyala J Agric Sci.* 2011;2(3):601–610.

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Plasmid-Mediated Carbapenem Resistance Among Clinical Klebsiella Pneumoniae Isolates in Thi-Qar, Iraq

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Authors' contribution: Zahraa Hassan Flaih – conceptualization, data curation, investigations, methods, project administration, resources, software, writing – original draft and writing – review & editing; Faten Naeem Abbas – conceptualization, data curation, methods, supervision, validation, writing – original draft and writing – review & editing; Tayseer Ali Talab – conceptualization, data curation, project administration, resources, supervision, validation, and writing – review & editing.
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Abstract

Introduction. Klebsiella pneumoniae is a nosocomial pathogen linked with multidrug resistance.
Purpose. This study aims to molecularly characterize plasmid-mediated carbapenem resistance in K. pneumoniae by detecting major carbapenemase genes (blaNDM, blaKPC, and blaIMP) and to compare phenotypic and molecular detection methods among clinical isolates sourced from medical centers in Thi-Qar, Iraq.
Materials and methods. A total of 300 specimens were collected. Identification of K. pneumoniae, as well as antimicrobial susceptibility testing, was performed by ViTeK 2 Compact; phenotypic detection was conducted by Rapidec Carba NP, after which the blaNDM, blaKPC, and blaIMP genes were detected by PCR.
Results. 130/300(43.3%) of K. pneumoniae isolates were identified. High resistance rates were observed against several antibiotics, particularly Ampicillin (73.846%), Ceftazidime (61.53%), Cefepime (57.69%), and Imipenem (43.08%). Rapidec Carba NP test revealed (41.54%) isolates that were carbapenem-producers. Molecular analysis revealed that the blaNDM gene was identified in (31.53%) of the isolates, the blaIMP gene (11.53%), and the blaKPC gene (0%). Notably, coexistence of two genes was detected in (4.6%) of isolates.
Conclusion. The outcomes from the use of the Rapidec Carba NP test showed a high level of agreement with molecular detection of the genes blaNDM and blaIMP, which proved the accuracy and sensitivity of the test.
Keywords: Klebsiella pneumoniae, carbapenem resistance, Rapidec Carba NP, blaIMP, blaNDM, blaKPC

■ INTRODUCTION

Klebsiella pneumoniae is recognized as an opportunistic Gram-negative bacterium. Clinically, multiple infectious conditions are frequently attributed to K. pneumoniae [1]. Horizontal gene transferable plasmids and integrons are the primary cause of the increase in multidrug-resistant (MDR) and carbapenem resistant K. pneumoniae

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(CRKP). These genetic elements accelerate the molecular evolution of resistance by efficient acquisition and spread of β-lactamase-associated resistance determinants [2]. Development of β-lactamases, especially carbapenemases, is the determinant of MDR in K. pneumoniae. Porin loss and efflux pump overexpression frequently work in concert to worsen carbapenem-resistant phenotypes; their clinical significance is attributed to their ability to hydrolyze carbapenems [3]. Ambler classification categorizes into four molecular categories. Within the Ambler classification, carbapenemase genes are distributed across different classes of β-lactamases. Klebsiella pneumoniae carbapenemase (KPC) belongs to class A, while New Delhi metallo-β-lactamases (NDM), Imipenemase (IMP), and Verona integron-encoded metallo-lactamases (VIM) belong to class B. Whereas, Oxacillinase-48(OXA-48) carbapenemases belong to Class D [4]. The clinical significance of CRKP has been formally recognized by the Centers for Disease Control and Prevention (CDC), which classified CRKP as an urgent public critical health issue due to its resistance to carbapenem [5].

This study aims to molecularly characterize plasmid-mediated carbapenem resistance in K. pneumoniae by detecting major carbapenemase genes (blaNDM, blaKPC, and blaIMP) and to compare phenotypic and molecular detection methods among clinical isolates collected from hospitals in Thi-Qar, Iraq.

■ MATERIALS AND METHODS

Collection of specimens

A total of 300 specimens were collected (from blood, wound swabs, urine, sputum, and leg abscesses). These were obtained from Nasiriya General Hospital in Thi-Qar between October 2025 and February 2026.

Isolation and identification

In the present study, 300 bacterial specimens were obtained by cultured on blood agar and MacConkey agar. Then confirmed by ViTeK 2 Compact system (bioMérieux, Marcy-L'Etoile, France).

Antibiotic susceptibility test (AST)

Antibiotic susceptibility testing for K. pneumoniae samples was accomplished by the ViTeK 2 Compact system using an AST card. 12 Antibiotics for 7 classes used in the current study were (Ampicillin, Ceftazidime, Cefepime, Trimethoprim-Sulfamethoxazole, Ceftriaxone, Amikacin, Piperacillin/Tazobactam, Gentamicin, Levofloxacin, Imipenem, Ertapenem, Ciprofloxacin).

Phenotypic analysis

The Rapidec Carba NP test (bioMérieux SA, Marcy l'Etoile, France) was used for the detection of carbapenemase through degradation of the β-lactam ring. In accordance with the manufacturer's guidelines, Mueller Hinton agar plates were used to grow isolated colonies of K. pneumoniae for 18–24 hours at 37 °C were suspended using a calibrated loop (10 µL). Then incubation at 37 °C for half an hour and again after two hours, if required. The development of yellow or orange is indicative of a positive result.

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Molecular analysis

Genetically, all K. pneumoniae isolates were evaluated for utilizing blaNDM, blaKPC, and blaIMP genes by using conventional PCR.

Genomic DNA extraction

Plasmid DNA extracted using SimEX™ Plasmid DNA Extraction Kit (SIM BIOLAB). The method is based on the principles of alkaline lysis and silica membrane spin column purification.

The extracted DNA was checked by the Nano drop system at wavelength 260 nm / 280 nm using the Nano Drop 2000 spectrophotometer [6].

Polymerase chain reaction (PCR)

PCR assays were performed for molecular analysis of carbapenemase-encoding genes (IMP, NDM, and KPC genes) using specific primers presented in Table 1. The total reaction volume was 20 µL formulated using master mix, primer pair (forward and reverse), DNA template, and nuclease-free water at volumes 10, 1, 3, 5 µL, respectively. The PCR amplification comprised five minutes of initial denaturation (at 95 °C), thirty-five amplification cycles, denaturation at 95 °C for half an hour, annealing at gene-specific temperatures (52 °C blaNDM, 56 °C blaKPC, and 55 °C blaIMP) for thirty seconds, with 1 minute extension at 72 °C; and completed process using a final extension at 72 °C lasting seven minutes [6].

Table 1
Primer sequences used to amplify genes

No.	Gene	Primer sequence 3' to 5'	Product size	Reference
1	blaKPC	F=CGTCTAGTTCTGCTGTCTTG R=CTTGTCATCCTTGTTAGGCG	798	[7]
2	blaIMP	F=TTGACACTCCATTTACDG R=GATYGAGAATTAAGCCACYCT	139	
3	blaNDM	F=ATGGAATTGCCCAATATTATGCAC R=TCAGCGCAGCTTGTCGGC	813	

The process of electrophoresis was applied for the separation of the PCR-generated products, which were examined on ethidium bromide-supplemented 2% agarose gel at a final concentration of 0.5 µg/mL. Electrophoresis occurred using 1X TAE buffer at 60V for eighty minutes. Each PCR run incorporated a positive control strain and a negative control using nuclease-free water [8].

Statistical analysis

Using SPSS version 26, statistical analysis of the current data was performed by Chi-square analysis at p-value (<0.05) [9].

Ethical approval

All subjects participating in this study were informed, and the consent form was verbally obtained from each one before sample collection. All procedures consistence with the ethical guidelines of Thi-Qar university, Iraq.

RESULTS

Isolation and identification of K. pneumoniae isolates

Out of 300 collected specimens, only 130 isolates (43.3%) were identified to be K. pneumoniae based on colony features (on blood agar and MacConkey agar). K. pneumoniae was isolated from different clinical samples, with sputum samples (33%) representing the most frequent source. Regarding patient demographics, 77 were male (59.23%), and 53 were female (40.769%), indicating male predominance. The male/female ratio was (1.45:1). Highest proportion was observed in the age group 41–50 (24.6%) years of age.

Antimicrobial resistance test of K. pneumoniae

Resistance was highest to Ampicillin, Ceftazidime, Cefepime, Trimethoprim-Sulfamethoxazole, Ceftriaxone, Amikacin, Ciprofloxacin, Piperacillin/Tazobactam, Gentamicin, Levofloxacin, Imipenem, Ertapenem with resistance rates of 73.85%, 61.53%, 57.69%, 55.38%, 53.85%, 50%, 50%, 46.15%, 46.15%, 46.15%, 43.08%, 43.08%, respectively as shown in figure 1.

Rapidec Carba NP test

Out of 130 K. pneumoniae isolates, 54 (41.54%) yielded positive results, which phenotypically confirmed as carbapenemase producers (figure 2A). 76 (58.46%) yielded negative results (figure 2B) as non-carbapenemase producers, which is depicted by no color change (Table 2).

Polymerase chain reaction (PCR)

The most prevalent genes were blaNDM, blaIMP, and blaKPC detected in 41/130 (31.53%), 15/130 (11.53%), 0/130 (0%) of the isolates, 6 isolates (4.6%) co-harbored two genes (blaNDM and blaIMP) respectively as shown in figures 3, 4 (Table 3).

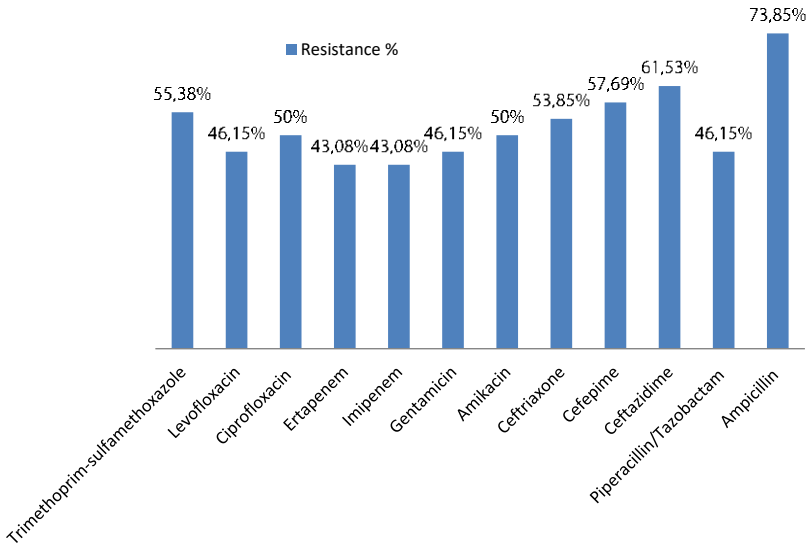
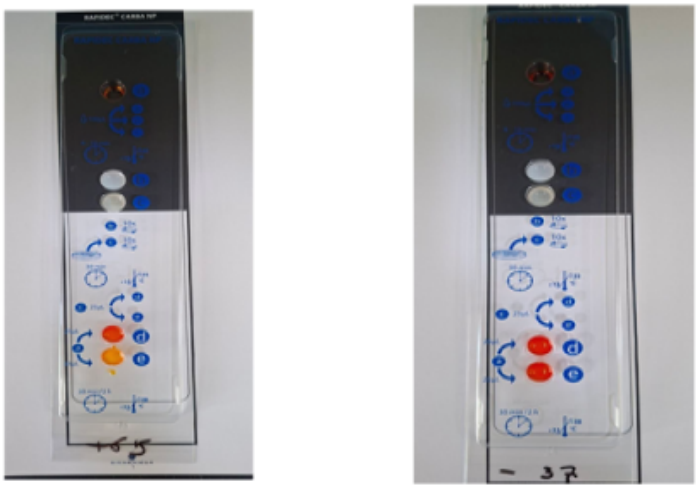


Fig. 1. Antibiotic resistance test



A B

Fig. 2. Rapides Carba NP Test: A – positive result; B – negative result

Table 2
Evaluation of agreement between AST and Rapides Carba NP results

K. pneumoniae (AST and Rapidec Carba NP)	No.	%	p. value
One Positive	2	1.54	<0.01
Both Positive	54	41.54	
Both Negative	74	56.92	
Total	130	100	

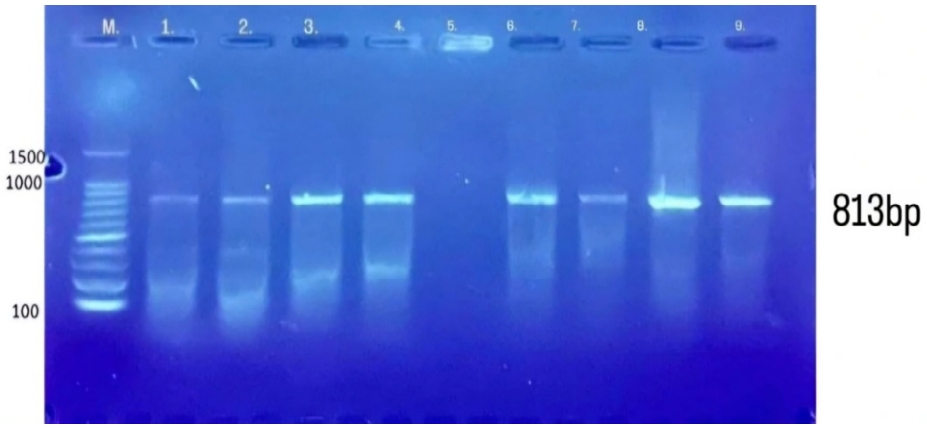


Fig. 3. Agarose gel electrophoresis (2%) of PCR amplification products of blaNDM gene (813 bp) in Klebsiella pneumoniae isolates (55 min at 60 v). M: represents ladder (DNA marker). Positive amplification of the blaNDM gene was observed in isolates 1–4, 6–9, while a negative isolate was 5

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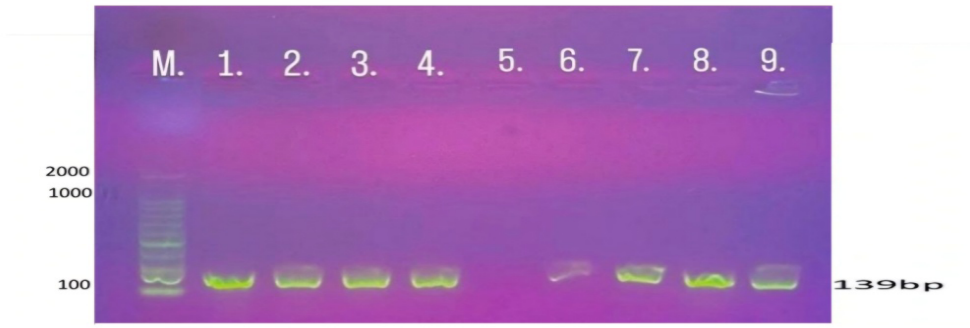

Fig. 4. Agarose gel electrophoresis (2%) of PCR amplification products of blaIMP gene (139 bp) in Klebsiella pneumoniae (55 min at 60 v). M: represents ladder (DNA marker). Positive amplification of the blaIMP gene was observed in isolates 1–4, 6–9, while a negative isolate was 5

Table 3
Frequency of carbapenemase genes

K. pneumoniae Genes Frequency (NDM, IMP, and KPC)	No.	%	p. value
Non-detect gene	80	61.53	<0.01
One gene	44	33.85	
Two genes	6	4.62	
Total	130	100	

Compare between phenotypic analysis and genotypic analysis

Concordance between the two techniques was observed to be high when identifying carbapenem-resistant strains. Most of the isolates that were positive by phenotypic testing had confirmation of the presence of the carbapenemase gene (blaNDM and blaIMP). This demonstrates that the phenotypic methods used were accurate and efficient when used

Table 4
Phenotypic and genotypic detection results

		Positive result		p. value
		No.	%	
Phenotypic	Antibiotic sensitive test	56	43.08	0.914
	Rapidec Carba NP test	54	41.5	
Genotypic	blaNDM	41	31.53	<0.01
	blaIMP	15	11.53	
	blaKPC	0	0	
	blaNDM + blaIMP	6	4.6	

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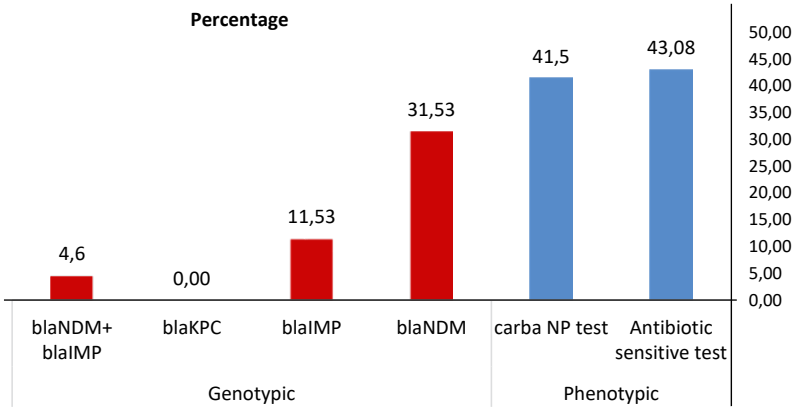

Fig. 5. Distribution of phenotypic and genotypic findings

as screening tools (Tables 4–6).

Table 5

Concordance between phenotypic assays and genotypic detection method

	Positive Genotype		Negative Genotype		p. value
	No.	%	No.	%	
Positive Phenotype	50	92.59	4	7.41	<0.01
Negative Phenotype	0	0.00	76	100	

Table 6

Comparative analysis of resistance detection methods and carbapenemase-encoding genes

	Positive		Negative		p. value
	No.	%	No.	%	
AST	56	43.076	74	56.92	0.114
Rapides Carba NP	54	41.54	76	58.46	0.054
NDM	41	31.54	89	68.46	<0.01
IMP	15	11.54	115	88.46	<0.01
KPC	0	0.00	130	100	–
CalX²=98.9 TabX²=9.49 DF=4 p. value <0.01					

■ DISCUSSION

The prevalence of CRKP has increased significantly. Resistance is mainly mediated by carbapenemase genes, including blaKPC, blaOXA, blaNDM, blaIMP, and blaVIM, as well as ESBL-associated mechanisms. These multidrug-resistant isolates exhibit resistance to multiple antibiotic classes, creating a major global concern due to the limited advancement of novel antimicrobial agents [10]. Therefore, the present study was conducted to investigate the prevalence carbapenemase genes among clinical isolates and comparison between phenotypic and genotypic analysis in province Thi-Qar, Iraq. The results in the present study demonstrated that 43.076% of K. pneumoniae were identified as carbapenem-resistant by antimicrobial susceptibility testing. This finding is consistent

with the results reported by [11] and reflects the continuing increase in carbapenem resistance among isolates. Whereas 54 (41.54%) were positive by the Rapidec Carba NP test. These findings are consistent with previous reports [12] and support the usefulness of phenotypic assays for the rapid identification of carbapenemase-producing isolates. Early identification of carbapenemase producers is crucial for optimizing antimicrobial therapy and implementing effective control measures for control of infection to limit nosocomial transmission [13].

The Rapides Carba NP test demonstrated 100% sensitivity and 95% specificity in the current study, confirming its reliability as a rapid screening method for routine laboratory use. These findings agree with previous investigations [14] and highlight the potential value of this assay in resource-limited settings, where rapid and cost-effective detection methods are essential for timely clinical decision-making. The high sensitivity of the Rapidec Carba NP test observed in the present study may be related to the predominance of Metallo-lactamases, particularly NDM, among the studied isolates, as these enzymes are effectively detected by this assay.

Molecular analysis revealed that blaNDM (31.54%) was the most prevalent carbapenemase gene among the studied isolates. This observation is consistent with several regional and international reports [15–17] that showed blaNDM high dominant rate (56.2%, 70.4%, 58.6%) respectively. But differs from others reported that blaNDM less prevalent [18]. The dissemination of blaNDM remains a critical healthcare concern, which has its capacity to enzymatically inactivate a wide spectrum of carbapenems. Its rapid spread has been strongly associated with international travel and inter-hospital patient transfer, facilitating cross-border transmission and accelerating the global expansion of carbapenem-resistant organisms [19].

In contrast, blaIMP was detected at a relatively low frequency (11.54%), a finding that agrees with previous studies in Iraq and globally [20, 21], showed that blaIMP less frequently distribution. But differs from others [22] that reported this gene higher prevalence rate in china. Geographic variation, variation in patterns antimicrobial consumption, local epidemiological characteristics, and infection-control strategies may contribute to the observed discrepancies between genes in my country [23]. Notably, blaKPC was not detected in the present study. This finding is consistent with local study in Al-Najaf city [16] and globally [24] and may suggest that blaKPC-producing strains have not yet become widely established in the studied region. However, the absence of blaKPC contrasts with findings reported elsewhere [25], that reported this gene higher prevalence of blaKPC in America, that emphasize the considerable geographic variability in the distribution of carbapenemase genes and the importance of continuous molecular surveillance. The lack of blaKPC detection among the examined isolates suggests that this gene may not be a primary determinant in carbapenem resistance in the study setting. In contrast, NDM-producing isolates were more prevalent, indicating that NDM-mediated resistance may represent the dominant carbapenem resistance mechanism in this region.

A small proportion of isolates (4.5%) co-harbored blaNDM and blaIMP genes. Although this frequency was relatively low, the coexistence of coexisting carbapenemase-encoding genes is epidemiologically important because it may enhance resistance levels and facilitate the emergence of highly resistant clones. Similar observations have been reported previously [26], highlighting the ongoing evolution and genetic diversification

of CRKP. In the current study four isolates yielded positive results by the Rapidec Carba NP test, despite being negative results for all targeted carbapenemase genes. This discrepancy may suggest the occurrence of additional carbapenemase genes that were not investigated in the present study. Similar discrepancies have been reported previously and have been attributed to factors such as porin deficiency, ESBL production in combination with permeability defects, or the presence of less frequently detected carbapenemase genes [27, 28]. Several limitations should be acknowledged. First, only three carbapenemase genes were screened and combined with no sequencing analysis, which limits comprehensive genetic characterization.

■ CONCLUSION

A considerable proportion of CRKP isolates were associated with Carbapenemase genes; the most predominant among these were blaNDM, with the widespread prevalence of Carbapenemases isolates. The strong concordance between the Rapidec Carba NP test and the molecular analysis (blaNDM and blaIMP) genes demonstrated the high sensitivity and accuracy of the assay, supporting its reliability as a rapid phenotypic assay for the identification of isolates that producing carbapenemases. The coexistence of resistance genes further highlights the need for combined phenotypic and molecular approaches for accurate detection and control.

■ REFERENCES

1. Hmood AH, Alammar MHM. Genotypic and phenotypic surveillance of multidrug resistance of hypermucoid K. pneumoniae among clinical isolates in AL Najaf City/Iraq. *Al-Kufa University Journal for Biology.* 2023;15(3):122–128.
2. Abbas FN. Molecular detection of bla(SHV), bla(IMP), and bla(VIM) β-lactamases genes among Klebsiella pneumoniae strains. *Indian Journal of Forensic Medicine & Toxicology.* 2021;15(2):2333–2338.
3. Li Y, Kumar S, Zhang L, et al. Characteristics of antibiotic resistance mechanisms and genes of Klebsiella pneumoniae. *Open Medicine (Poland).* 2023;18(1):1–12.
4. Alvisi G, Curtoni A, Fonnesu R, et al. Epidemiology and genetic traits of carbapenemase-producing Enterobacterales: a global threat to human health. *Antibiotics.* 2025;14(2):141.
5. Mohammadpour D, Memar MY, Ebrahimzadeh Leylabadlo H, et al. Carbapenem-resistant Klebsiella pneumoniae: a comprehensive review of phenotypic and genotypic methods for detection. *The Microbe.* 2025;6:100246.
6. García-Alegría AM, Anduro-Corona I, Pérez-Martínez CJ, et al. Quantification of DNA through the NanoDrop spectrophotometer: methodological validation using standard reference material and Sprague Dawley rat and human DNA. *International Journal of Analytical Chemistry.* 2020;2020:8896738.
7. Hamad ST, Ghaim KK, Al-Lawi AA. Prevalence of carbapenemase genes in Klebsiella pneumoniae isolates from patients with urinary tract infections in Baghdad hospitals. *Iraqi Journal of Biotechnology.* 2022;21(1):102–114.
8. Green MR, Sambrook J. Analysis of DNA by agarose gel electrophoresis. *Cold Spring Harbor Protocols.* 2019;2019(1):1–5.
9. Okab HF, Khazaal H, Majed MM. Estimation of Immunological and Physiological Parameters in Diabetic Patients with COVID-19. *Rev Bionatura.* 2023;8(2):8–11.
10. Sisay A, Kumie G, Gashaw Y, et al. Prevalence of genes encoding carbapenem-resistance in Klebsiella pneumoniae recovered from clinical samples in Africa: systematic review and meta-analysis. *BMC Infectious Diseases.* 2025;25.
11. Sharma S, Sharma A. Detection of NDM, VIM, IMP metallo-β-lactamase genes in Klebsiella pneumoniae isolated from clinical samples at a tertiary care centre: a study from Southern Rajasthan. *Global Journal for Research Analysis.* 2024.
12. Vamsi SK, Moorthy RS, Hemiliamma MN, et al. Phenotypic and genotypic detection of carbapenemase production among gram negative bacteria isolated from hospital acquired infections. *Saudi Medical Journal.* 2022;43(3):236–243.
13. Bedos JP, Daikos G, Dodgson AR, et al. Early identification and optimal management of carbapenem-resistant Gram-negative infection. *J Hosp Infect.* 2021;108:158–167.
14. Anani HAA, El Madbouly AA, Althoqapy AA, et al. Genotypic and phenotypic screening of carbapenemase-mediated resistance in Klebsiella pneumoniae clinical isolates: A hospital-based study. *Al-Azhar Univ J Virus Res Stud.* 2021;3(1):1–12.
15. Gandor NHM, Amr GE, Eldin Algammal SMS, Ahmed AA. Characterization of carbapenem-resistant K. pneumoniae isolated from intensive care units of Zagazig University Hospitals. *Antibiotics.* 2022;11(8):1108.
16. Mohanna ZA, Al-Yasseen AK. Distribution of carbapenemase genes among carbapenem-resistant Klebsiella pneumoniae isolates from the patients in Najaf, Iraq. *Biomedical and Biotechnology Research Journal.* 2024;8(3):297–304.
17. Neupane B, Devkota MD, Pokhrel BM, et al. Phenotypic characteristics and carbapenemase genes in Klebsiella pneumoniae from patients at Upendra Devkota Memorial National Institute of Neurological and Allied Sciences, Kathmandu, Nepal. *BMC Infectious Diseases.* 2025;25(1):1119.
18. Aguilar-Ancori E, Marin-Carrasco M, Campo-Pfuyo LI, et al. Identification of pandemic ST147, ESBL-type β-lactamases, carbapenemases, and

virulence factors in Klebsiella pneumoniae isolated from southern Peru. *Sci Rep.* 2025;15.
19. Loconsole D, Sallustio A, Sacco D, et al. Genomic surveillance of carbapenem-resistant Klebsiella pneumoniae reveals a prolonged outbreak of extensively drug-resistant ST147 NDM-1 during the COVID-19 pandemic in the Apulia region (Southern Italy). *Journal of Global Antimicrobial Resistance.* 2024;36:260–266.
20. Hussein NH. Emergence of NDM-1 among carbapenem-resistant Klebsiella pneumoniae in Iraqi hospitals. *Acta Microbiol Immunol Hung.* 2018;65(2):211–227.
21. Kaboré B, Ouédraogo GA, Ouédraogo HS, et al. Identification and phylogenetic analysis of carbapenemase genes from clinical strains of Klebsiella pneumoniae. *Journal of Infection in Developing Countries.* 2024;18(9):1387–1393.
22. Xue Y, Zhang D, Du S, et al. Molecular epidemiological characteristics of blaIMP-4-carrying Klebsiella pneumoniae ST-11 in hospitalized patients. *Infection and Drug Resistance.* 2025;18:171–184.
23. Al-Hasani HMH, Al-Rubaye DS, Abdelhameed A. The emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) in Iraqi clinical isolates of Escherichia coli. *J Popul Ther Clin Pharmacol.* 2023;30(5):469–482.
24. Homkaew A, Wongsuk T, Boonsilp S, et al. Diversity of carbapenemase genes among carbapenem-resistant Klebsiella pneumoniae in a tertiary care hospital in Bangkok, Thailand. *Acta Microbiol Immunol Hung.* 2025.
25. Tesfa T, Mitiku H, Edae M, Assefa N. Prevalence and incidence of carbapenem-resistant K. pneumoniae colonization: systematic review and meta-analysis. *Systematic Reviews.* 2022;11.
26. Osama D, El-Mahallawy H, Mansour MT, et al. Molecular characterization of carbapenemase-producing Klebsiella pneumoniae isolated from Egyptian pediatric cancer patients including a strain with a rare gene-combination of β-lactamases. *Infection and Drug Resistance.* 2021;14:335–348.
27. Zhang A, Wang X, Liang X, al. Performance evaluation of diagnostic assays for detection and classification of carbapenemase-producing organisms. *Antibiotics (Basel).* 2021;10(12):1457.
28. Pruss A, Skierska A, Kwiatkowski P, et al. Comparative analysis of selected methods of carbapenemase determination among clinical Klebsiella pneumoniae. *PLOS ONE.* 2025;20(2):e0318852.

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The Patterns of Antimicrobial Resistance in Bacterial Causes of Lower Respiratory Tract Infections: A Public Health Viewpoint from Sputum Surveillance Data

Conflict of interest: nothing to declare.
Authors' contribution: Adil Fadil – conceptualization, data curation, software, supervision, validation, visualization, and writing – review & editing; Raad Luty – conceptualization, validation, visualization, writing – original draft and writing – review & editing; Jasim Najm – conceptualization, methods, project administration, resources, software, supervision, validation, writing – original draft and writing – review & editing; Safa Mahdi – conceptualization, data curation, project administration, resources, validation, visualization, writing – original draft and writing – review & editing.
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Abstract

Introduction. Lower respiratory tract infections (LRTIs), such as community-acquired pneumonia (CAP), and hospital-acquired pneumonia (HAP), are a major source of morbidity and mortality worldwide. Antimicrobial resistance (AMR) is becoming more prevalent, which is compromising the effectiveness of empirical antibiotic therapy; therefore, it is important to investigate antibiotic sensitivity pattern of the bacterial agents for optimal antibacterial prescription.
Materials and methods. This hospital-based cross-sectional observational study aimed to evaluate the antimicrobial resistance patterns of bacterial agents causing LRTI through a retrospective data analysis of sputum culture results for both types of bacterial identifications and antibiotic susceptibility profiles among 189 patients who presented with lower respiratory tract infections (LRTI) and were admitted to the largest hospital in southern Iraq from August, 2018, until November, 2019.
Results. The Gram-positive bacteria Streptococcus pneumoniae was the first most frequent causative isolate, whereas Gram-negative bacteria Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii were the first, second, and third most frequent causative isolates respectively. Imipenem, ertapenem, ceftriaxone, and cefotaxime had high susceptibility rates against S. pneumoniae. Meropenem, imipenem, and amikacin had high susceptibility rates against K. pnumoniae and P. aeruginosa, while no antibiotic has a high susceptibility rate against A. baumannii.
Conclusion. The major causative isolate S. pneumoniae shows high antimicrobial resistance rates against important antibiotics including amoxicillin, amoxicillin/clavulanic acid, ciprofloxacin, levofloxacin, ofloxacin, cefepime, and vancomycin, tetracycline, linezolid, and clindamycin which call for reconsidering them for treatment of LRTI, while the Gram-negative isolates show high antimicrobial resistance rates against the majority of the tested antibiotics.

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Keywords: lower respiratory tract infections, antimicrobial resistance, antibiotic susceptibility, Streptococcus pneumoniae, Klebsiella pneumoniae

■ INTRODUCTION

Lower respiratory tract infections accounted for 2·5 million in 2023, with the greatest burden falling on people 70 years of age and older and children under five. Staphylococcus aureus (271 000 deaths, or 10·9%), Klebsiella pneumoniae (228 000 deaths, or 9·1%), and Streptococcus pneumoniae (634 000 deaths, or 25·3% of all LRI deaths) remained to be the leading cause of LRTIs mortality worldwide [1]. Globally, lower respiratory tract infections pose a significant challenge to healthcare systems [2]. Bacterial lower respiratory tract infections can cause everything from minor flare-ups of chronic bronchitis to potentially fatal pneumonias, and the epidemiological information on potential infections and local resistance profiles is typically used to inform the choice of empirical antibiotic therapy [3]. However, many conventional empirical regimens have become ineffective because to the quick and widespread spread of antimicrobial resistance (AMR), which has resulted in treatment failures, longer hospital stays, higher healthcare expenses, and increased mortality [4, 5]. It is noteworthy that prevalent bacteria associated in LRTI, especially S. pneumoniae, frequently exhibit continuously rising rates of antibiotic resistance [6], however another feature in this regard on a worldwide scale is the notable regional variation in the rates of resistance to these empirically used antibiotics, which is mostly due to the manner in which antibiotics are used [7]. This rapid emergence of bacterial antibiotics resistance threatens the lives of millions over the world and increases patient hospital stay with increasing the management cost [8]. The irrational stewardship and lack of regulations regarding antibiotics use that result in increased consumption and promote overuse are the principal causes for increased bacterial antibiotic resistance [9]. Here it is good to mention that this pattern of irrational stewardship is widely distributed in many developing countries as it is estimated that the global proportion of non-prescription antibiotics supply by community pharmacies is 62% [10].

In fact, health systems around the world face a significant challenge in managing LRTIs, which calls for striking a balance between providing efficient individual patient care and maintaining the population's access to antibiotics [11]. AMR surveillance is recognized by the World Health Organization (WHO) as a fundamental component of its Global Action Plan [12], and a crucial and useful source of local epidemiological intelligence in this situation is routine sputum culture and sensitivity data from both outpatient departments and inpatient wards. Aggregated results offer a crucial evidence base for developing local treatment guidelines, tracking resistance trends, and assessing the effects of public health interventions, such as immunization campaigns and antibiotic stewardship programs, despite certain limitations, such as specimen quality [13].

The prevalence of community-acquired resistance is shown in data from sputum samples of patients who visit primary care or outpatient clinics, and the Important trends consist of:

- Streptococcus pneumoniae: The effectiveness of first-line medications like amoxicillin and azithromycin, which are advised by numerous guidelines, is directly challenged by surveillance, which continuously reveals large and regionally varying rates of

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penicillin and macrolide non-susceptibility [14, 15]. This pattern emphasizes how crucial pneumococcal conjugate vaccination programs are to public health as a means of lowering the incidence of illness and resistant strains [16].

- Haemophilus influenzae: The use of amoxicillin monotherapy is compromised by the prevalence of beta-lactamase-producing strains and increasingly BLNAR (beta-lactamase-negative ampicillin-resistant) strains, requiring the empirical use of beta-lactam/beta-lactamase inhibitor combinations (such as amoxicillin-clavulanate) in high prevalence areas [17]. These findings serve to reduce needless selection pressure in the population by refuting the empirical use of broader-spectrum medications such as respiratory fluoroquinolones or third-generation cephalosporins for simple CAP [18, 19]. A unique and highly resistant ecology is revealed in sputum cultures from hospitalized patients, especially those with healthcare-associated pneumonia (HCAP), HAP, or VAP, this ecology is influenced by previous antibiotic exposure, comorbidities, and hospital settings [20].
- Staphylococcus aureus (MRSA): Isolating MRSA from sputum frequently indicates previous medical contact and requires switching to glycopeptide or linezolid medication, which affects treatment plans and infection control strategies [21]. In hospital-onset LRTIs, MDR Gram-Negative Bacilli (GNB) are the primary public health concern.
- ESBL-Producing Enterobacterales: Most cephalosporins are ineffective due to the high incidence of ESBLs in K. pneumoniae and E. coli isolates from inpatient sputum, which leads to increasing usage of carbapenems and subsequent selection for carbapenem resistance [22, 23]. Both Acinetobacter baumannii and Pseudomonas aeruginosa: Late-onset VAP is frequently caused by several environmental infections. High frequencies of MDR and extensively drug-resistant (XDR) phenotypes are frequently found in sputum monitoring, which leaves few effective treatment options and results in subpar outcomes [24]. Cross-transmission is made easier by their persistence in the hospital setting.

Microbial species that are typically susceptible to a specific antibiotic may generate more resistant strains by spontaneous mutation or the acquisition of novel resistance genes followed by selection. The genetic changes cause a number of mechanisms that underlie resistance, including enzyme inactivation, alteration of the antibiotic target site, and lower drug accumulation due to greater efflux or decreased permeability. Drug-sensitive rivals are eliminated by antibiotics, allowing resistant microorganisms to spread due to natural selection [4, 25].

This study's primary goal is to evaluate the antimicrobial resistance patterns of bacterial pathogens that cause lower respiratory tract infections in patients who visit a tertiary care hospital. To do this, the automated VITEK® 2 compact system – a precise method recently used in microbiological laboratories – is used to determine the distribution and antibiotic susceptibility profile of causative bacterial agents in patients with LRTI. The majority of clinical isolates of both Gram-positive and Gram-negative bacteria can be accurately identified and tested for antibiotic sensitivity using the new automated VITEK® 2 compact system. Reduced turnaround times, better specimen handling, increased quality control, reproducibility, and the capacity to monitor outcomes are additional benefits of the system [26].

■ MATERIALS AND METHODS

Study design

This a hospital – based cross-sectional observational study was carried out in a hospital to evaluate patterns of antibiotic resistance in bacterial pathogens that cause lower respiratory tract infections (LRTIs). The study was conducted over a period of one and a half yea], from August 2021 to November 2022. The study population comprised patients of all age groups and both sexes who attended the tertiary care hospital during the study period with clinical features suggestive of lower respiratory tract infection.

Inclusion Criteria include; Patients clinically diagnosed with lower respiratory tract infection, Patients whose respiratory samples (sputum) yielded significant bacterial growth, and both inpatients and outpatients.

Exclusion Criteria include; Patients who had received antibiotic therapy within 48–72 hours prior to sample collection, inadequate or contaminated respiratory samples, and duplicate samples from the same patient. All eligible respiratory samples received in the microbiology laboratory during the study period were included, a consecutive sampling technique was used, and it was 189 patients with lower respiratory tract infection.

Following Al-Basrah Teaching Hospital's consent, approval was received from the Scientific Committee of the Center of Training and Human Development/Research Unit in the Health Directorate of Al-Basrah Governorate (Date 10-July-2019/NO. 375). Sputum samples were gathered and sent to the microbiology lab in a wide-mouth sterile container. A pre-designed and pre-tested proforma was used to gather data. The following details were noted: Clinical information, kind of respiratory sample, sociodemographic information (age, sex), and relevant risk factors (smoking status, comorbidities such diabetes mellitus or chronic lung disease, past antibiotic usage when available).

Sample culture and bacterial identification

A loop-full of each sample was streaked onto blood agar, MacConkey agar, and chocolate agar to inoculate the sputum and bronchial wash samples. The inoculum was then equally spread around the plate. Blood and MacConkey agars were incubated in an aerobic atmosphere at 37 °C for 24 hr., while Chocolate agar was incubated in a 10% Co2 incubator at 37 °C for 24 hr. for identification of facultative anaerobic isolates. Mycobacterium tuberculosis and anaerobic species were not included in the present study. The growth of the aerobically incubated bacteria was identified by the characteristics and morphology of the colony, and Gram staining.

Significant bacterial growth was defined as a colony count of at least 100,000 colony forming units (CFU)/ml. GPB and GNB genus and species tests were identified using the VITEK® 2 compact system (bioMérieux S.A., France) protocols using the following kits: VITEK® 2 GN Reference 21341, VITEK® 2 GP Reference 21342. Since S. pneumoniae is not included in the automated VITEK® 2 compact system, it was diagnosed manually using culture, gram stain, and morphology.

Antibiotics susceptibility

AST-P641, AST-N326, and AST-N327 cards were used in the VITEK® 2 compact system (bioMérieux, Frace) to determine antibiotic susceptibility testing. Piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, amikacin, gentamicin, netilmicin, tobramycin, ciprofloxacin, levofloxacin, tetracycline, tigecycline, trimethoprim/

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sulfamethoxazole, fosfomycin, nitrofurantoin, benzylpenicillin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, and fusidic acid were the antibiotics that VITEK® 2 cards. The antibiotic susceptibility test for S. pneumoniae was conducted using the Kirby-Bauer disc diffusion method. The 2015 Clinical and Laboratory Standards Institute (CLSI) standards were used to interpret the results. Antibiotic-resistant or intermediate-susceptible isolates were regarded as non-susceptible.

Data analysis

Data were entered into Microsoft Excel and analyzed using SPSS, version 23. Descriptive statistics were mentions as frequencies expressed as number and percentages.

■ RESULTS

Patients' characteristics

One hundred and eighty-nine sputum samples – 120 from males and 69 from females – showed notable bacterial growth in the current investigation. Table 1 lists the characteristics of the patients. 62.9% of LRTI cases are in young and middle-aged individuals (25–64 years). Elderly patients (≥65 years) make up 16.8% of the total, while pediatric patients (0–14 years) make up 18%. Patients in the 45–64 age range account for the largest percentage of all LRTI cases (28.6%), followed by those in the 25–44 age range (24.8%).

Table 1
Sex and age distribution of lower respiratory tract infection patients

	No. (%)
Sex	
Female	69 (36.5)
Male	120 (63.5)
Gender distribution	
1–14	10 (5.3)
15–24	18 (9.5)
25–44	53 (28.0)
45–64	66 (34.9)
65+	42 (22.2)

Species distribution

Table 2 displays the distribution of LRTI species. Of the 189 bacterial isolates, Gram-positive was predominant 130 (2 genera, 4 species) and 59 were Gram-negative (9 genera, 12 species). Gram positive bacteria accounted for over half (68.8%) of all cultured sputum, while Gram negative bacteria accounted for 31.2%, according to the septum cultures. Gram-negative isolates K. pneumoniae (9.5%) and P. aeruginosa (8.5%) were the second and third most common isolates found in sputum cultures, respectively, S. pneumoniae is predominant pathogen accounted for 65.1% of all isolates of the LRTI. In addition to other isolates, such as E. coli, Staphylococcus species, and streptococcus species, which are present in small percentages. This table also shows that while Gram positive bacteria were more predominant in terms of number, Gram negative bacteria were more in terms of species.

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Table 2
Distribution of bacteria isolated from sputum culture

Bacterial isolates	No. (%)
Gram-positive bacteria	130 (68.8)
Streptococcus pneumoniae	123 (65.1)
Staphylococcus aureus	3 (1.6)
staphylococcus epidermidis	2 (1.1)
Staphylococcus haemolyticus	2 (1.1)
Gram-negative bacteria	59 (31.2)
Klebsiella pneumoniae	18 (9.5)
Pseudomonas aeruginosa	16 (8.5)
Pseudomonas luteola	1 (0.5)
Acinetobacter baumannii	8 (4.2)
Acinetobacter caloaceticus	1 (0.5)
Eschericia coli	6 (3.2)
Protus hauseri	2 (1.1)
Proteus penneri	2 (1.1)
Enterobacter cloacae	2 (1.1)
Serratia marcescens	1 (0.5)
Raoultella ornithinolytica	1 (0.5)
Cedecea lapagei	1 (0.5)

Antibiotics susceptibility

The antibiotic susceptibility of the Gram-positive isolates is displayed in Table 3. The first most common isolate of S. pneumonia has high susceptibility rates (>70%) to ertapenem, imipenem, ceftriaxone, cefotaxime, doxycycline, and rifampicin, but high resistance rates (>30–40%) to penicillin, amoxicillin/clavulanic acid, ciprofloxacin, levofloxacin, ofloxacin, cefepime, vancomycin tetracycline, trimethoprim/sulfamethoxazole. While, ciprofloxacin, levofloxacin, teicoplanin, tigecycline, linezolid, fucidin, nitrofurantoin, fosfomycin, and trimethoprim/sulfamethoxazole are all highly susceptible to S. aureus, the second most common Gram-positive strain. Tables (4 and 5) showed the Gram-negative isolates' susceptibility to antibiotics, the most common Gram-negative isolate, K. pneumonia, exhibits high susceptibility rates to amikacin, imipenem, and meropenem but high resistance rates (40–100%) to the remaining antibiotics in the cassette panel reserved for Gram-negative isolates.

In the cassette panel reserved for Gram-negative isolates, P. aeruginosa, the second most common isolate, exhibits high sensitivity rates to ceftazidime and amikacin (60 and 66.7%, respectively), but high resistance rates (60–100%) to the remaining medicines. The third most common Gram-negative isolate, A. baumannii, was solely sensitive to imipenem. There were 18 (100%), 4 (25%), 5 (62.5%), and 4 (66.7) isolates of K. pneumoniae, P. aeruginosa, A. baumannii, and E. coli that were resistant to several drugs. Since not all of the antibiotics identified by the group of worldwide specialists were examined, it is impossible to quantify the extensive drug resistance and pan drug.

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Table 3
Antibiotics resistance percentage R (R%) of Gram-positive isolates[a]

Antibiotics	Streptococcus pneumoniae (123)		Staphylococcus aureus (3)		Staphylococcus haemolyticus (2)	
Benzylpenicillin	–	–	2/2	(100)	2/2	(100)
Penicillin	11/33	(33.3)	1/1	(100)	–	–
Amoxicillin	41/91	(45.1)	–	–	–	–
Amoxicillin/ Clavulonic acid	10/29	(34.5)	–	–	–	–
Ciprofloxacin	21/41	(51.2)	1/3	(33.3)	2/2	(100)
Levofloxacin	41/90	(45.6)	1/3	(33.3)	1/1	(100)
Ofloxacin	75/140	(53.6)	–	–	–	–
Imipenem	8/80	(10.0)	–	–	–	–
Ertapenem	10/54	(18.5)	–	–	–	–
Ceftriaxone	19/116	(16.4)	–	–	–	–
Cefotaxim	13/63	(20.6)	–	–	–	–
Cefepime	52/107	(48.6)	–	–	–	–
Vancomycin	30/73	(41.1)	2/3	(66.7)	–	–
Teicoplanin	–	–	0/3	0	–	–
Tetracycline	59/88	(67.0)	1/2	(50.0)	2/2	(100)
Erythromycin	–	–	3/3	(100)	1/1	(100)
Tigecycline	–	–	0/3	0	0/1	0
Linezolid	14/29	(48.3%)	1/3	(33.3)	1/2	(50.0)
Clindamycin	10/20	(50.0%)	3/3	(100.0)	2/2	(100)
Fucidin	–	–	2/3	(66.7)	2/2	(100)
Nitrofurantoin	–	–	1/3	(33.3)	0/1	0
Fosfomycin	–	–	1/3	(33.3)	2/2	(100)
Doxacycline	16/58	(27.6)	–	–	–	–
Rifampicin	15/107	(14.0)	–	–	–	–
Trimethoprim/Sulfamethoxazole	97/99	(98.0)	0/2	0	1/2	(50.0)

Note: [a] Staphylococcus epidermidis not applied for antibiotics.

Table 4
Antibiotics resistance percentage R (R%) of Gram-negative isolates[a]

Antibiotics	Klebsiella pneumoniae (18)		Pseudomonas aeruginosa (16)		Pseudomonas luteola (1)		Acinetobacter baumannii (8)		Acinetobacter caloaceticus (1)		Escherichia coli (6)	
Piperacillin	5/7	(71.4)	10/14	(71.4)	1	(100%)	3/3	100	–		–	
Piperacillin/ Tazobactam	9/17	(52.9)	9/13	(69.2)	1	(100%)	4/4	100	0	0	4/6	(66.6)
Ceftazidime	18/18	(100)	8/14	(40.0)	0	0	8/8	100	0	0	6/6	(100)
Cefepime	5/9	(55.9)	7/13	(53.8)	1	(100%)	3/3	100	–		5/5	(100)
Aztreonam	8/8	(100)	6/14	(42.9)	1	(100%)	3/3	100	–		6/6	(100)
Meropenem	6/15	(40.0)	6/12	(50.0)	0	0	2/4	50	0	0	0/6	0
Imipenem	6/18	(33.3)	3/12	(25.0)	0	0	5/6	83.3	0	0	0/6	0
Ciprofloxacin	10/18	(55.6)	6/11	(54.5)	0	0	4/6	66.7	0	0	5/6	(83.3)
Levofloxacin	5/6	(83.3)	5/11	(45.4)	0	0	2/4	50.0	–		4/6	(66.7)

Trimetho-prim/Sulfa-methoxazole	16/17	(94.1)	7/9	(77.8)	0	0	6/6	100	0	0	4/6	(66.7)
Tetracycline	6/8	(75.0)	13/14	(92.9)	0	0	3/3	100	–		4/5	(80.0)
Tigecycline	–		12/12	(100)	0	0	–		–		–	
Gentamicin	10/18	(55.5%)	6/13	(46.1)	0	0	4/8	50	0	0	3/6	(50.0)
Amikacin	7/18	(38.9)	5/15	(33.3)	0	0	4/6	66.7	0	0	1/6	(16.7)
Tobramycin	6/8	(75.0)	7/13	(53.9)	0	0	–		–		3/6	(50.0)
Netilmicin	6/7	(85.7)	6/12	(50.0)	0	0	–		–		2/4	(50.0)
Colistin	–		2/15	(13.3)	1	(100%)	–		–		–	

Note: [a] Proteus penneri not applied for antibiotics.

Table 5
Antibiotics resistance percentage R (R %) of Gram-negative isolates

Antibiotics	Protus hauseri (2)		Enterobacter cloacae (2)		Serratia marc-escens (1)		Raoultella orni-thinolytica (1)		Cedecea lapagei (1)	
Piperacillin	–		–		0	0	0	0	–	
Piperacillin/Tazobactam	0/2	0	1/2	(50.0)	0	0	0	0	1	(100)
Ceftazidime	2/2	(100)	1/2	(50.0)	0	0	0	0	1	(100)
Cefepime	–		–		0	0	0	0	–	
Aztreonam	–		–		0	0	0	0	–	
Meropenem	2/2	(100)	0/2	0	0	0	0	0	0	0
Imipenem	2/2	(100)	0/2	0	0	0	0	0	0	0
Ciprofloxacin	0/2	0	0/2	0	0	0	0	0	0	0
Levofloxacin	–		–		0	0	0	0	–	
Trimethoprim/ Sulfamethoxazole	2/2	(100)	0/2	0	0	0	0	0	0	0
Tetracycline	–		–		0	0	0	0	–	
Tigecycline	–		–		0	0	0	0	0	0
Gentamicin	0/2	0	0/2	0	0	0	0	0	0	0
Amikacin	0/2	0	0/2	0	0	0	0	0	0	0
Tobramycin	–		–		0	0	0	0	–	
Netilmicin	–		–		0	0	0	0	–	
Colistin	–		–		1	(100)	0	0	–	

■ DISCUSSION

The present study shows that S. pneumoniae, K. pneumonia, and P. aeruginosa were the first, second, and third most predominant causative isolates respectively that identified in the sputum clinical samples since this study did not determine the type of infection whether it was acquired from the hospital or the community, so there was a diversity of the causative isolates that have been recorded in the other studies in a variable frequency depending on the setting of the infections whether hospital or community-acquired infections and upon geographical locations [2, 7, 27, 28]. The antibiotics susceptibility of S. pneumonia isolates shows high resistance rates to amoxicillin, amoxicillin/ clavulanic acid, ciprofloxacin levofloxacin, ofloxacin cefepime vancomycin tetracycline, and trimethoprim/sulfamethoxazole. This resistance rate is somewhat similar or slightly higher regarding some antibiotics in comparison with studies conducted recently in

other countries in different regions of the world as Japan [28], Switzerland [29], and Turkey [30]. K. pneumoniae shows higher resistance rates regarding beta-lactam antibiotics and quinolones in comparison with other studies carried out in Jordan [31], and Malaysia [32]. It shows comparable high resistance rates to piperacillin/tazobactam, ceftazidime, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, and nitrofurantoin in comparison to other studies [30, 33]. P. aeruginosa isolates showed high resistance rates (40–60%) to all antibiotics tested but imipenem, amikacin, and colistin.

This pattern of resistance is higher than those found in studies carried in Japan [27], Swiss [29], and Turkey [30]. A. baumannii which constitute 4.2% of the causative isolates showed a serious antibiotics susceptibility pattern represented by high resistance rates to all but imipenem antibiotics (100% for 8 antibiotics) a result that comes in accordance with other studies which show that this species is resistant to the most antibiotics and difficult to treated and a similar finding was recorded recently in Iraq [34, 35].

■ CONCLUSION

The high level of bacterial antibiotic resistance may be caused by irrational use, improper prescription, and over-the-counter availability of antibiotics, which is a common practice in the Al-Basrah Governorate. Since nearly all antibiotics are sold in private pharmacies without a medical prescription, local and national health authorities must implement regulations and policies regarding the handling and prescription of antibiotics in order to reduce infection-related morbidities and mortality, prevent serious clinical effects and consequences, and preserve the effectiveness of various antibiotic categories against pathogenic bacteria.

■ REFERENCES

1. Sirota Sarah Brooke, et al. Global burden of lower respiratory infections and aetiologies, 1990–2023: a systematic analysis for the Global Burden of Disease Study 2023. *The Lancet Infectious Diseases,* 2026 Apr;26(4):343-361. 10.1016/S1473-3099(25)00689-9
2. Torres A., Cilloniz C., Niederman M.S., et al. Pneumonia. *Nature Reviews Disease Primers,* 2021;7(1):25.
3. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. *American Journal of Respiratory and Critical Care Medicine,* 2019;200(7):e45–e67.
4. *World Health Organization. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2022*. Geneva: WHO; 2022.
5. Centers for Disease Control and Prevention (U.S.). Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.
6. Cherazard R, Epstein M, Doan T-L, et al. Antimicrobial Resistant Streptococcus pneumoniae: Prevalence, Mechanisms, and Clinical Implications. *Am J Ther.* 2017;24:e361–e369.
7. Iroezindu MO, Chima EI, Isiguzo GC, et al. Sputum bacteriology and antibiotic sensitivity patterns of community-acquired pneumonia in hospitalized adult patients in Nigeria: a 5-year multicentre retrospective study. *Scand J Infect Dis.* 2014 46:875–887.
8. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P T.* 2015;40:277–83.
9. Zaman S Bin, Hussain MA, Nye R, et al. A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus.* 2017;9:e1403.
10. Auta A, Hadi MA, Oga E et al. Global access to antibiotics without prescription in community pharmacies: A systematic review and meta-analysis. *J Infect.* 2019;78:818.
11. Laxminarayan R, Matsoso P, Pant S, et al. Access to effective antimicrobials: a worldwide challenge. *Lancet*. 2016;387(10014):168–175.
12. World Health Organization. *Global Action Plan on Antimicrobial Resistance*. Geneva: World Health Organization; 2015.
13. O'Brien TF, Stelling J. Integrated Multilevel Surveillance of the World's Infecting Microbes and Their Resistance to Antimicrobial Agents. *Clin Microbiol Rev*. 2011;24(2):281–295.
14. The WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR). *Critically Important Antimicrobials for Human Medicine, 6th Revision*. Geneva: World Health Organization; 2017.
15. Sader HS, Mendes RE, Le J, et al. Antimicrobial Susceptibility of Streptococcus pneumoniae from North America, Europe, Latin America, and the Asia-Pacific Region: Results From 20 Years of the SENTRY Antimicrobial Surveillance Program (1997–2016). *Open Forum Infect Dis*. 2019;6(Suppl 1):S14–S23.
16. Rückinger S, van der Linden M, Reinert RR, et al. Reduction in the incidence of invasive pneumococcal disease after general vaccination with 7-valent pneumococcal conjugate vaccine in Germany. *Vaccine*. 2009;27(31):4136–4141.

17. Andrzejczuk S, Kosikowska U, Chwiejczak E, et al. Prevalence of Resistance to β-Lactam Antibiotics and *bla* Genes Among Commensal *Haemophilus parainfluenzae* Isolates from Respiratory Microbiota in Poland. *Microorganisms*. 2019;7:427.
18. Goossens H, Ferech M, Vander Stichele R, Elseviers M; ESAC Project Group. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet*. 2005;365(9459):579–587.
19. Costelloe C, Metcalfe C, Lovering A, et al. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ*. 2010;340:c2096.
20. Cillóniz C, Martín-Loeches I, García-Vidal C, et al. Microbial Etiology of Pneumonia: Epidemiology, Diagnosis and Resistance Patterns. *Int J Mol Sci*. 2016;17(12):2120.
21. Kallen AJ, Mu Y, Bulens S, et al. Health Care–Associated Invasive MRSA Infections, 2005–2008. *JAMA*. 2010;304(6):641–648.
22. Jean SS, Chang YC, Lin WC, et al. Epidemiology, treatment, and prevention of nosocomial bacterial pneumonia. *J Clin Med*. 2020;9(1):275.
23. Pitout JDD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis*. 2008;8(3):159–166.
24. Bassetti M, Vena A, Croxatto A, et al. How to manage Pseudomonas aeruginosa infections. *Drugs Context*. 2018;7:212527.
25. Cynthia NC, Bruce D. Fisher RAH. Lippincott's Illustrated Reviews: Microbiology. 3rd ed. Lippincott Williams & Wilkins. Philadelphia; 2013.
26. Donay JL, Mathieu D, Fernandes P, et al. Evaluation of the Automated Phoenix System for Potential Routine Use in the Clinical Microbiology Laboratory. *J Clin Microbiol.* 2004;42:1542–1546.
27. Yanagihara K, Kadota J, Aoki N, et al. Nationwide surveillance of bacterial respiratory pathogens conducted by the surveillance committee of Japanese Society of Chemotherapy, the Japanese Association for Infectious Diseases, and the Japanese Society for Clinical Microbiology in 2010: General v. *J Infect Chemother.* 2015;21:410–420.
28. Djordjevic ZM, Folic MM, Jankovic SM. Distribution and antibiotic susceptibility of pathogens isolated from adults with hospital-acquired and ventilator-associated pneumonia in intensive care unit. *J Infect Public Health.* 2017;10:740–744.
29. Sommerstein R, Atkinson A, Lo Priore EF, et al. Characterizing non-linear effects of hospitalisation duration on antimicrobial resistance in respiratory isolates: an analysis of a prospective nationwide surveillance system. *Clin Microbiol Infect.* 2018;24:45–52.
30. Uskudar Guclu A, Altay Kocak A, Akcil Ok M, et al., Respiratory Study Group. Antibacterial Resistance in Lower Respiratory Tract Bacterial Pathogens: A Multicenter Analysis from Turkey. *J Infect Dev Ctries.* 2021;15:254–262.
31. Sallam M. Trends in Antimicrobial Drug Resistance of *Streptococcus pneumoniae* Isolates at Jordan University Hospital (2000–2018). *Antibiotics.* 2019;8:41.
32. Arushothy R, Ahmad N, Amran F, et al. Pneumococcal serotype distribution and antibiotic susceptibility in Malaysia: A four-year study (2014–2017) on invasive paediatric isolates. *Int J Infect Dis.* 2019;80:129–133.
33. Al-Zalabani A, AlThobyane OA, Alshehri AH, et al. Prevalence of Klebsiella pneumoniae Antibiotic Resistance in Medina, Saudi Arabia, 2014–2018. *Cureus.* 2020;12:e9714.
34. Lob SH, Hoban DJ, Sahm DF, Badal RE. Regional differences and trends in antimicrobial susceptibility of *Acinetobacter baumannii*. *Int J Antimicrob Agents.* 2016;47:317–323.
35. Chawsheen MA, Al-Naqshbandi AA, Abdulqader HH. Bacterial Profile and Antimicrobial Susceptibility of Isolates Recovered from Lower Respiratory Tract Infection for Patients in Rizgary Hospital, Erbil. *ARO-THE Sci J KOYA Univ.* 2020;8:64–70.

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Transcriptomics Analysis Reveals Oxaloacetate-TBK1 Axis as a Critical Target for RSV NS1-Mediated Immune Evasion

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Abstract

Introduction. Respiratory Syncytial Virus (RSV) causes severe respiratory infections and evades long-term immunity through poorly understood mechanisms. The viral Non-Structural Protein 1 (NS1), a known interferon antagonist, also reprograms host cellular metabolism.
Purpose. To investigates the hypothesis that NS1 induces a metabolic collapse of the innate immune system by depleting mitochondrial oxaloacetate (OAA), a critical metabolite required for the MDH1-mediated induction of the antiviral TBK1 signaling cascade.
Materials and methods. By integrating transcriptomic analysis of human epithelial cells infected with mitochondrial-incompetent NS1 mutants (NS1-Y125A and NS1-L132A/L133A) with metabolic rescue experiments using cell-permeable oxaloacetate. The study demonstrates a novel axis of immune regulation. Mitochondrial-incompetent NS1 mutants (Y125A and L132A/L133A) fail to suppress immune responses, upregulating IFNB1, IFNLs, CXCL11, and IDO1 and activating TLR and RIG-I-like receptor signaling pathways. Cell-permeable oxaloacetate (DEOAA) restores antiviral gene signatures, mimicking the genetic restoration of immunity in a separate viral infection model.
Results. Integrated analysis demonstrates a highly significant transcriptional convergence (p=5.436e−37) between NS1 mutation and oxaloacetate supplementation, centered on a core antiviral activation module. The predictive strength of the metabolic sensor MDH1 and kinase TBK1 in a machine learning model mechanically links mitochondrial oxaloacetate availability to innate immune activation. RSV NS1 protein suppresses innate immunity by depleting mitochondrial oxaloacetate, acting as a metabolic checkpoint inhibitor.
Conclusion. This depletion disrupts the MDH1-TBK1 signaling axis, preventing effective viral detection by host cells. Restoring oxaloacetate levels or metabolic balance reactivates antiviral defenses, suggesting a potential therapeutic strategy for respiratory viral infections.

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Keywords: respiratory syncytial virus (RSV), non-structural protein 1 (NS1), oxaloacetate, immunometabolism, innate immunity

■ INTRODUCTION

Respiratory Syncytial Virus (RSV) is a primary cause of severe lower respiratory tract infections, including bronchiolitis and pneumonia, in infants and young children [1, 2]. RSV causes mortality in elderly and immunocompromised adults at rates comparable to influenza. Despite over six decades of intensive research since its discovery, the medical community lacks a licensed vaccine for the general pediatric population, and therapeutic interventions remain restricted to prophylactic monoclonal antibodies like palivizumab and nirsevimab [3–7]. Hosts fail to develop durable, sterilizing immunity to RSV, which impedes vaccine development and characterizes the virus's pathogenesis. Frequent reinfection with RSV throughout life indicates the virus evades detection and subverts immunological memory [8, 9].

The non-structural protein 1 (NS1) drives this evasion strategy. The Pneumoviridae-specific protein NS1 antagonizes the host interferon (IFN) response and is the most abundantly transcribed viral gene early in infection. The original model for NS1 function involved direct protein-protein interference. It is well-established that NS1 forms a degradative complex with STAT2 to cripple the JAK-STAT pathway and binds to the mitochondrial antiviral-signaling protein (MAVS) to inhibit RIG-I signaling [10–12]. Steric inhibition alone cannot explain NS1-mediated cellular reprogramming. Antiviral responses are energetically expensive, requiring specific metabolites as signaling molecules [13–16]. We hypothesize RSV NS1 suppresses innate immunity by physically blocking immune receptors and collapsing the system's metabolism. We examined how the tricarboxylic acid (TCA) cycle intermediate oxaloacetate (OAA) regulates the interferon response. Cytosolic OAA is required to bind and dimerize the metabolic sensor MDH1 (Malate Dehydrogenase 1), which in turn scaffolds the transcription factor ETS2 to drive the expression of TBK1, the obligatory kinase for IRF3 activation. Without this metabolic signal, the immune system is effectively suppressed upstream of the canonical signaling cascade [16, 17].

Evidence suggests that RSV induces a state of metabolic decoy, where the cell is hypermetabolic and glycolytically active to fuel viral replication, yet selectively starved of the metabolites required for immunity. We suggest that NS1 induces this starvation. By localizing mitochondrion, NS1 induces mitochondrial fragmentation and loss of membrane potential. This disruption is predicted to stall the Malate-Aspartate Shuttle, trapping oxaloacetate equivalents within the mitochondrial matrix and depleting the cytosolic pool necessary for MDH1-mediated immune sensing [18, 19].

Here, we show this novel axis of immune evasion. We combine transcriptomic analysis of human epithelial cells infected with mitochondrial-incompetent NS1 mutants (NS1-Y125A and NS1-L132A/L133A) with metabolic rescue experiments using cell-permeable oxaloacetate. Failure of NS1 mutants to localize to mitochondria prevents innate immunity suppression, inducing a strong antiviral gene signature. Oxaloacetate supplementation during wild-type viral infection phenocopied the genetic rescue, restoring interferon signaling. NS1 is a metabolic checkpoint inhibitor. Reversing viral immune suppression by restoring metabolic homeostasis is therefore a new host-directed therapeutic strategy.

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■ MATERIALS AND METHODS

Cohorts and differential expression analysis

The study used raw RNA-sequencing count data from GEO accession GSE309353. We infected human epithelial cells with WT RSV, RSV NS1-Y125A, and RSV NS1-L132A/L133A (n = 6 per group). We validated our results with expression data from GSE297098, an experiment on influenza A (H1N1) infected cells treated with diethyl-oxaloacetate (DEOAA) or vehicle. Analyzed the data in R (v4.4.2). We normalized raw counts from the RSV dataset (GSE309353) using DESeq2 [20]. A linear model controlled for donor and infection status. Differential expression analysis was performed using the Wald test. Genes were considered significantly differentially expressed (DEGs) if they satisfied a Benjamin-Hochberg adjusted p-value (padj) < 0.05 and an absolute log2 fold change (|log2FC|) > 1.0. This threshold identified biologically significant changes.

Pathway and gene set enrichment analysis

We analyzed the biological functions of DEGs with Gene Ontology (GO) Biological Process enrichment using the GSEApy Python library. We analyzed gene sets using GO_Biological_Process_2023 and KEGG_2021_Human. Specific focus was placed on immune signaling pathways. For targeted pathway analysis, we retrieved gene sets for Cytokine-cytokine receptor interaction, Toll-like receptor signaling, and others from the KEGG database. Module scores were calculated by Z-score transforming the expression matrix and computing the mean Z-score for all genes within a given pathway for each sample.

Cross-dataset integration and predictive modeling

To assess the concordance between the RSV NS1 mutant phenotype and the oxaloacetate-supplemented phenotype, we merged datasets based on common gene symbols. We constructed a quadrant scatter plot of Log2 Fold Changes (Log2FC) to visualize concordantly regulated genes. Furthermore, we employed a Random Forest classifier (scikit-learn/randomForest package) to test if a compact signature of 18 metabolic and antiviral genes could predict the treatment status (DEOAA vs Vehicle) in independent samples. The model was trained with 500 trees, and feature importance was extracted using the Mean Decrease in Gini impurity metric.

■ RESULTS

RSV NS1 mutants exhibit a distinct transcriptional profile and restore host immunity

To test our hypothesis that mitochondrial interaction is key to NS1's immunosuppressive function, we first analyzed the transcriptomic landscape of human epithelial cells infected with wild-type (WT) RSV or one of two NS1 mutants (NS1Y125A and NS1L132A/L133A) previously implicated in altering NS1 function. PCA of the transcriptomes revealed a striking separation of samples based on the infection condition (Fig. 1). While WT RSV-infected cells were observed to cluster differently than the cells infected with either of the two NS1 mutants clustered together, away from the WT group. Point mutations in NS1 alter host cell transcription in a reproducible pattern distinct from WT virus.

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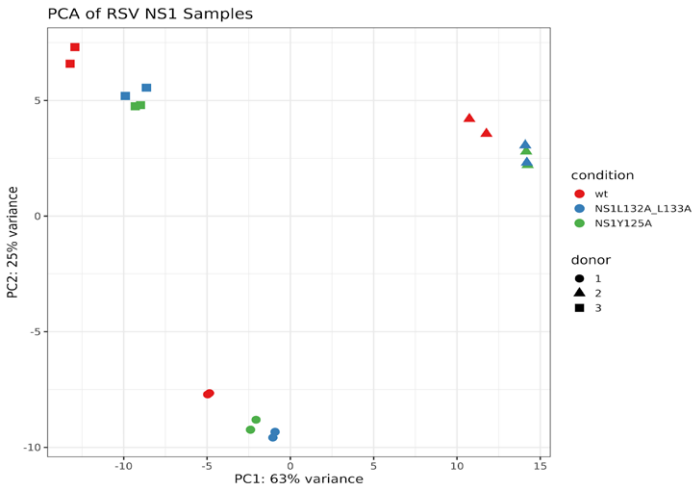

Fig. 1. PCA of RSV NS1 Samples PCA separated samples by gene expression. Colors denote conditions (wt, NS1L132A_L133A, NS1Y125A) and shapes denote donors. PC1 separated samples by viral genotype and PC2 by infection status. NS1 mutations drive most of the transcriptional variance in infected samples

The study identified genes driving the separation through differential expression analysis. The NS1Y125A mutation upregulated canonical antiviral genes relative to WT RSV (Fig. 2). Interferons IFNB1 and IFNL1-3 showed significant upregulation, along with interferon-stimulated genes (ISGs) such as the chemokine CXCL11 and the antiviral effector IDO1. Mutant NS1 fails to suppress the host interferon response.

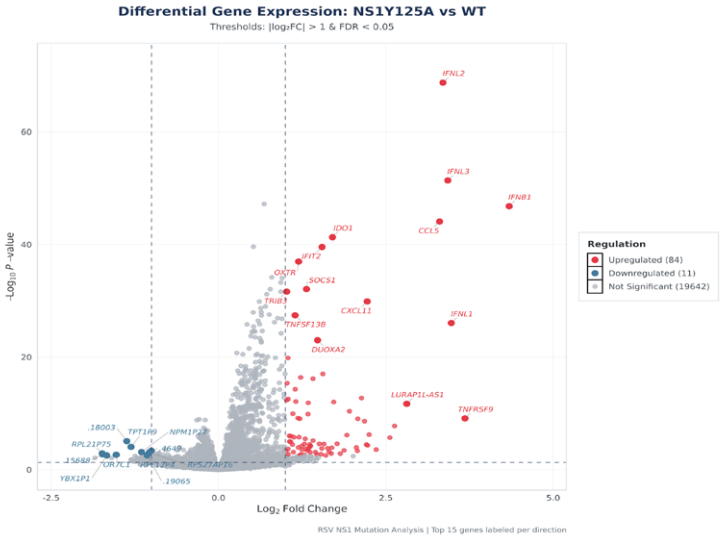

Fig. 2. Differential Gene Expression: NS1Y125A vs WT we plotted Log2 fold change against the -Log10 P-value for all genes. Red denotes genes significantly upregulated in the NS1Y125A mutant versus WT RSV. The induction of interferons and chemokines restores the host immune response

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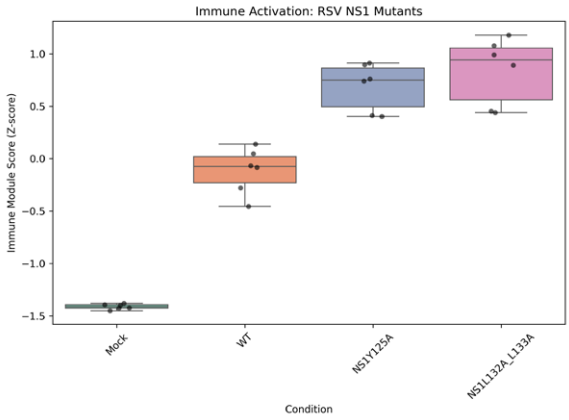

Fig. 3. Immune Activation: RSV NS1 Mutants Immune module scores by infection condition. WT RSV infection suppresses the immune score compared to mock, whereas infections with NS1Y125A and NS1L132A/L133A mutants significantly increase it

We scored immune activation across all conditions using a curated set of antiviral genes. WT RSV-infected cells showed a lower immune score than mock-infected cells (Fig. 3). In stark contrast, infection with either the NS1Y125A or NS1L132A/L133A mutant virus led to a dramatic and significant restoration of the immune activation score, elevating it to levels far exceeding those in WT-infected cells. As hypothesized, the NS1 mutants failed to suppress host immunity.

Mutation of NS1 reverses suppression of key antiviral pathways

We defined the restored immune signature's biological context through pathway enrichment analysis of genes upregulated in NS1 mutants relative to WT. Host antiviral response pathways showed enrichment (Fig. 4). The most significantly enriched term was "Cytokine-cytokine receptor interaction" (Adjusted P = 2.11e-09), followed by a cascade of innate immunity pathways including "Toll-like receptor signaling pathway" (Adjusted P = 9.68e-04), "Cytosolic DNA-sensing pathway" (Adjusted P = 9.68e-04), "RIG-I-like receptor signaling pathway" (Adjusted P = 1.11e-02), and "JAK-STAT signaling pathway" (Adjusted P = 6.07e-03). The coordinated upregulation of these pathways confirms that the NS1 mutations lead to a broad-based de-repression of the cellular machinery designed to detect and combat viral infection. Conversely, no biological processes were significantly enriched among the few genes downregulated by the mutants, suggesting that the primary effect of these mutations is the loss of a suppressive function.

Oxaloacetate Supplementation Phenocopies the NS1 Mutant Immune Profile

Our central hypothesis posits that NS1's immunosuppressive activity is mediated through the depletion of OAA. To test this, we leveraged an independent transcriptomic dataset (GSE297098) where cells infected with influenza virus were treated with a vehicle or DEOAA, a cell-permeable form of OAA. We first calculated the same immune activation score used for the RSV dataset. In vehicle-treated cells, influenza infection induced only a modest immune response (Fig. 5). However, supplementation with DEOAA during

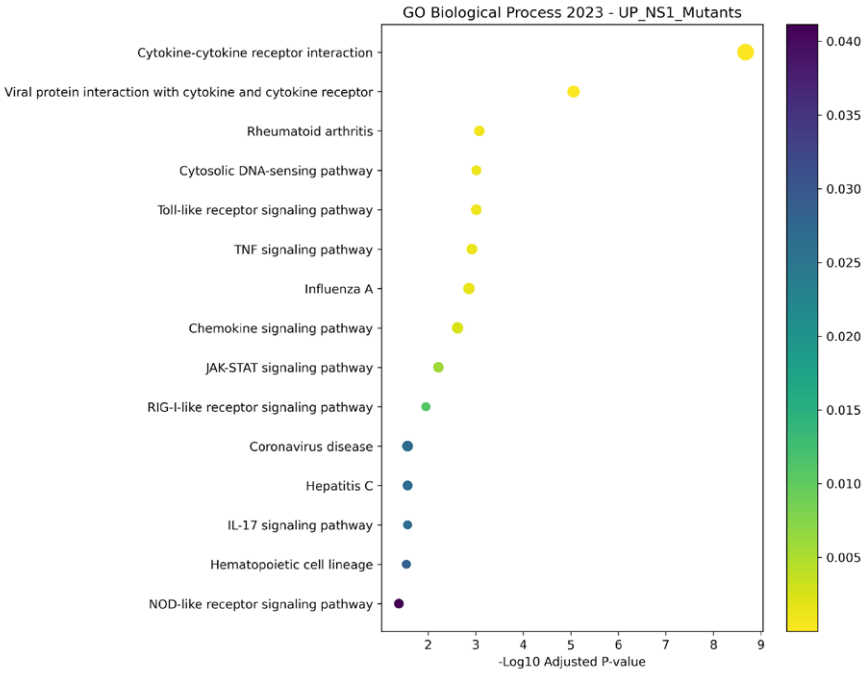

Fig. 4. GO Biological Process 2023 – UP_NS1_Mutants Dot plot showing the top enriched biological pathways for genes upregulated in NS1 mutants compared to WT RSV. The x-axis represents the -Log10 Adjusted P-value, and the color scale indicates the significance. Pathways related to cytokine signaling, viral sensing, and innate immunity are highly enriched

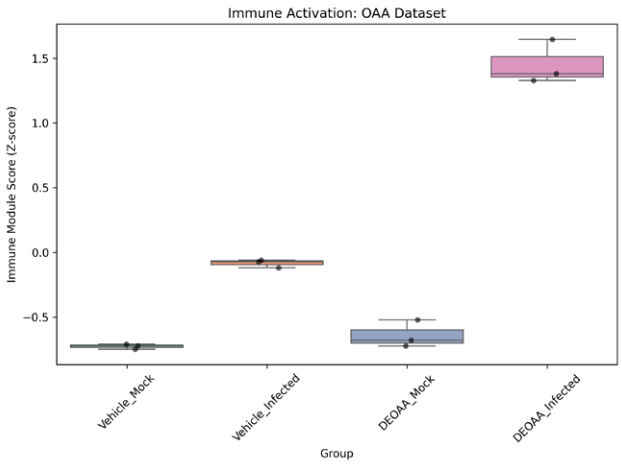

Fig. 5. Immune Activation: OAA Dataset Boxplot of the immune module score in cells treated with vehicle or diethyl-oxaloacetate (DEOAA) and either mock-infected or infected with influenza virus. DEOAA supplementation significantly boosts the immune score during infection compared to vehicle-treated infected cells

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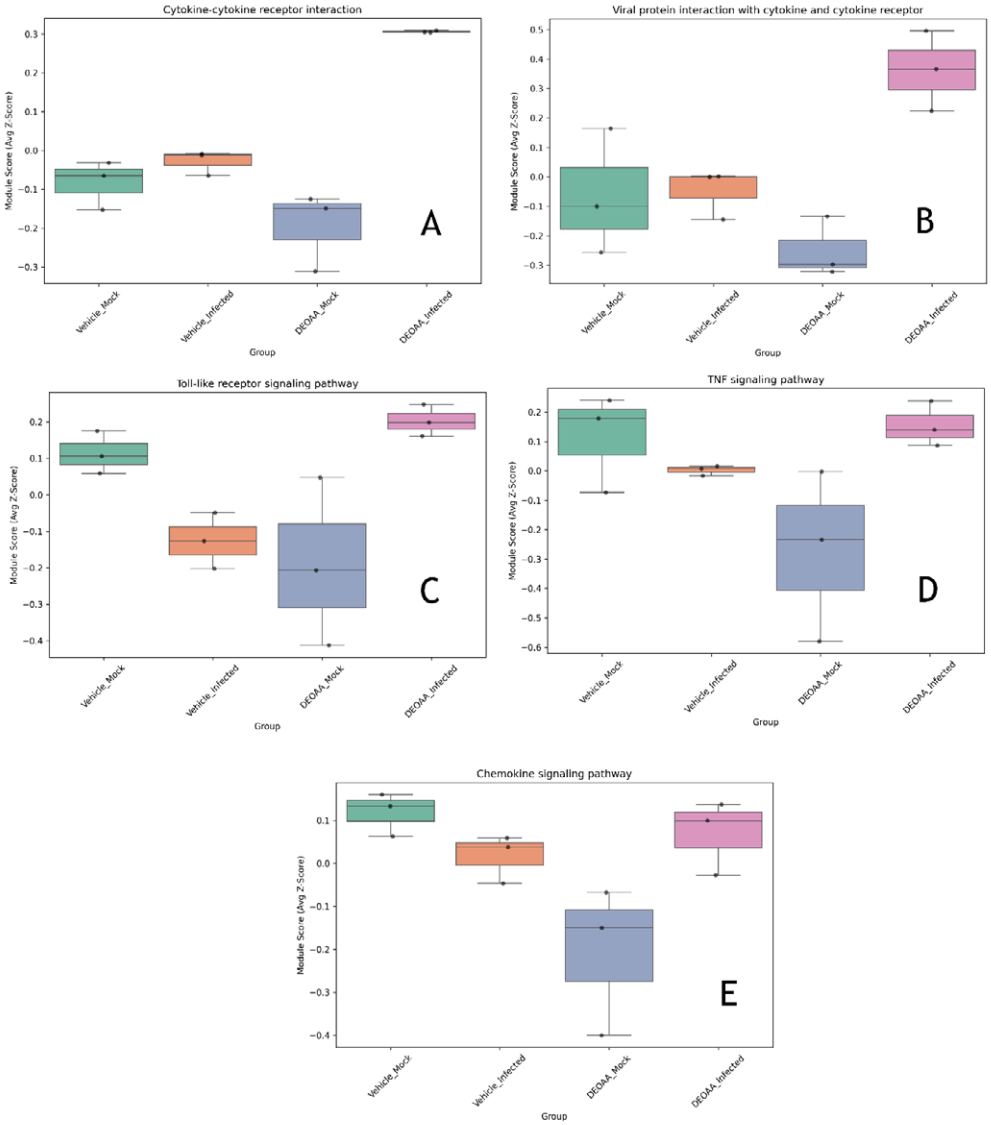

Fig. 6. A – Cytokine-cytokine receptor interaction Module scores measure cytokine-cytokine receptor pathway activity. Infected cells treated with DEOAA showed significantly higher pathway activity than controls. B – Viral protein interaction with cytokine and cytokine receptor, the score measures viral protein interaction pathway activity. Supplementing DEOAA during infection increases pathway activity. C – Toll-like receptor signaling pathway Module score for the Toll-like receptor signaling pathway. DEOAA increases pathway activity during infection. D – TNF signaling pathway We scored TNF signaling pathway activity. Supplying DEOAA during infection increases pathway activity. E – Chemokine signaling pathway the module score quantified Chemokine signaling pathway activity. DEOAA activity showed a non-significant increase during infection

infection resulted in a striking and potentiation of the immune score, demonstrating that restoring OAA levels can rescue a suppressed antiviral state.

We then examined the effect of DEOAA on specific immune pathways. In agreement with the broad immune activation, DEOAA supplementation during infection led to a significant increase in the activity of several key pathways compared to vehicle-treated infected cells, including "Cytokine-cytokine receptor interaction" (P = 0.00005, Fig. 6A), "Viral protein interaction with cytokine and cytokine receptor" (P = 0.011, Fig. 6B), "Toll-like receptor signaling pathway" (P = 0.003026, Fig. 6C), and "TNF signaling pathway" (P = 0.028, Fig. 6D). The "Chemokine signaling pathway" showed a similar trend but did not reach statistical significance (P = 0.42, Fig. 6E). (Fig. 6F) shows the extent of antiviral pathway induction by OAA. The metabolic rescue phenotypically matches the genetic rescue in NS1 mutants, which confirms our hypothesis.

Fig. 7. Quadrants of Gene Regulation Gene expression changes (Log2FC) following RSV NS1 mutant infection (x-axis) are plotted against changes following OAA supplementation (y-axis). The plot shows a significant positive correlation, with many key antiviral genes located in the "Concordant Upregulation" quadrant (Up-Up, red)

Transcriptomic signatures converge between NS1 mutation and OAA supplementation

We compared the transcriptional effects of NS1 mutation and OAA supplementation by integrating both datasets. Gene expression log2 fold changes in the NS1 mutant versus WT correlated with those from OAA supplementation versus vehicle (Fig. 7). The two experimental systems shared a transcriptional response, correlating positively ($R = 0.085$, $p = 5.436e\text{-}37$). The "Concordant Upregulation" quadrant was densely populated with critical antiviral genes, including IFNB1, IFNL1, IFNL2, IFNL3, CCL5, CXCL11, IFIH1, and TBK1, which are upregulated in both NS1 mutant infections and OAA-supplemented conditions (Fig. 7). A shared mechanism drives immunity restoration in NS1 mutants and its rescue by OAA supplementation.

A Core Metabolic and Antiviral Gene Signature Links the Two Perturbation States

We next defined a core signature of 18 key metabolic and antiviral genes, including TCA cycle enzymes (MDH1, CS) and central antiviral signaling molecules (TBK1, IFNB1). Hierarchical clustering of the RSV dataset using this signature perfectly segregated the WT-infected samples from the NS1 mutant-infected samples (Fig. 8). The WT condition was characterized by suppressed expression of interferons and ISGs, while the mutants displayed a mirror image of potent induction.

In the OAA dataset, activity of the 18-gene signature increased in DEOAA-treated infected cells compared to vehicle controls (Fig. 9A). OAA supplementation during infection also induced a module score of genes upregulated by NS1 mutants, suggesting a functional overlap (Fig. 9B).

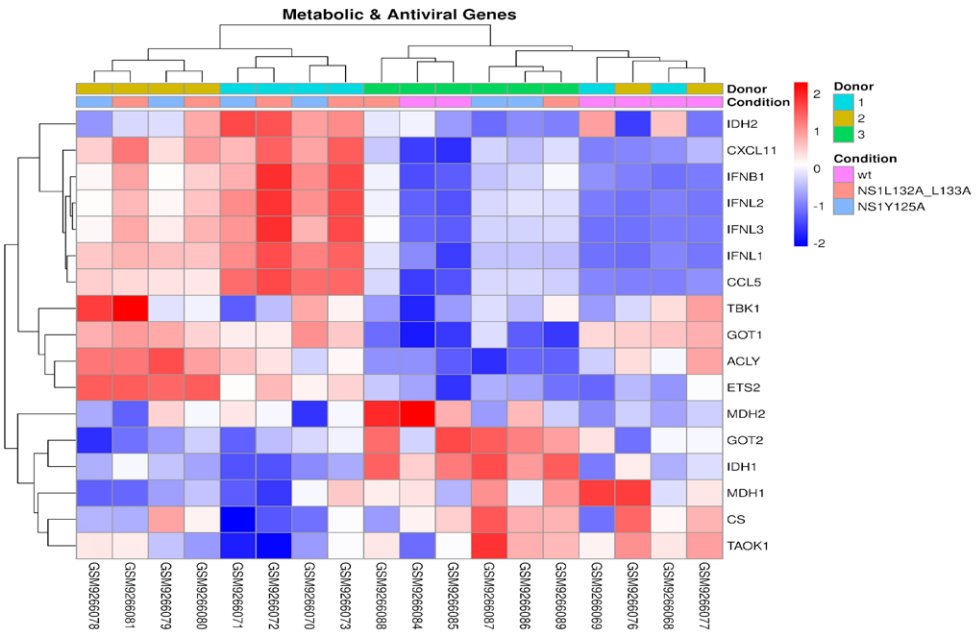

Fig. 8. Metabolic & Antiviral Genes Heatmap showing the expression of a curated 18 gene signature across RSV-infected samples. The NS1 mutant activates an immune signature suppressed during wild-type infection

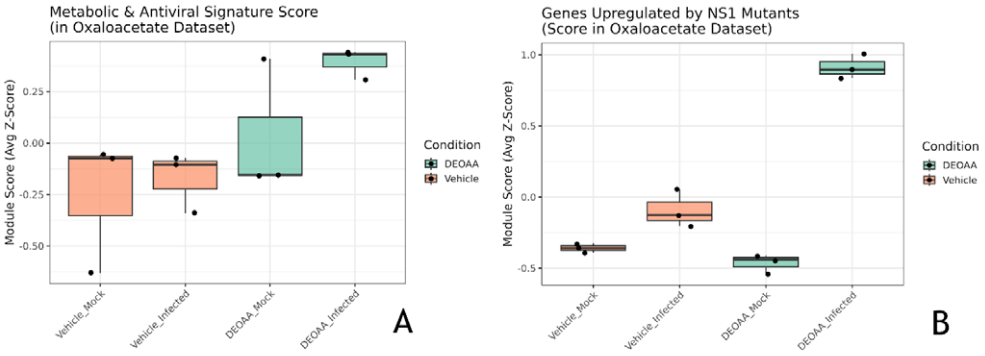

Fig. 9. A – Metabolic & Antiviral Signature Score An 18-gene signature scores metabolic and antiviral activity in the OAA dataset. Infected cells treated with DEOAA have a significantly higher score, similar to cells with NS1 mutations. B – Genes Upregulated by NS1 Mutants Module score for genes upregulated by NS1 mutants, scored in the OAA dataset. OAA supplementation during infection strongly induces this gene set, demonstrating a shared transcriptional program

Finally, to test the predictive power of this signature, we trained a Random Forest classifier to distinguish between DEOAA- and vehicle-treated samples using only the expression of these 18 genes. The model performed with high accuracy, and an analysis of feature importance revealed that both antivirals signaling molecules (TBK1, IFIH1, CCL5) and metabolic enzymes (MDH1, IDH2, CS) were critical for the classification (Fig. 10). The prominence of MDH1 (Mean Decrease Gini = 0.26), an enzyme directly involved in OAA metabolism, as the second most important feature provides a powerful predictive link between the metabolic state and the antiviral phenotype. Finally, the shortlisted markers are summarized in Table, whereas other data points of this study are presented in Supplementary Table.

Shortlisted biomarkers identified through concordance and discordance along with machine learning

Gene Symbol	RSV Log2FC	OAA Log2FC	ML Importance (Gini)	Regulation
IFNB1	4.34	2.25	0.168	Up-Up
IFNL1	3.48	2.97	0.175	Up-Up
IFNL2	3.35	0.2	0.045	Up-Up
IFNL3	3.43	0.54	0.154	Up-Up
CXCL11	2.22	2.96	0.165	Up-Up
CCL5	3.3	1.53	0.184	Up-Up
IFIH1	0.68	1.25	0.199	Up-Up
TBK1	0.04	3.12	0.305	Up-Up
MDH1	–0.07	–0.14	0.261	Down-Down
IDH2	0.04	–0.18	0.161	Up-Down
CS	–0.02	–0.08	0.093	Down-Down
IDO1	1.7	3.23	0	Up-Up
RSAD2	0.98	1.93	0	Up-Up
STAT1	0.59	0.61	0	Up-Up
MX1	0.7	0.87	0	Up-Up

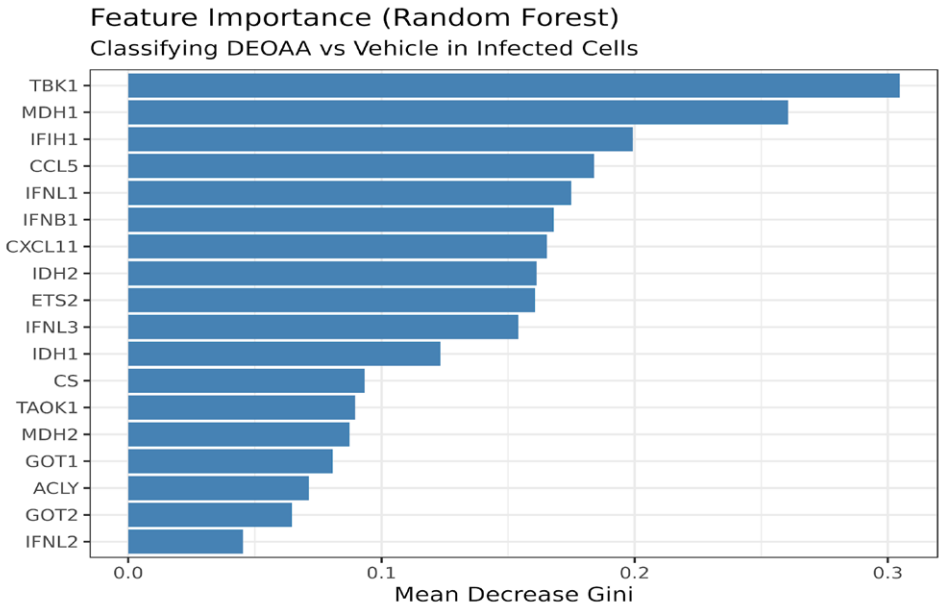

Fig. 10. Feature Importance (Random Forest) Bar chart showing the feature importance (Mean Decrease in Gini) from a Random Forest model trained to classify DEOAA vs. vehicle treatment in infected cells. Both antiviral (TBK1, IFIH1) and metabolic (MDH1) genes are identified as key predictors

■ DISCUSSION

This study demonstrates that the immunosuppressive potency of NS1 may rely critically on its ability to hijack mitochondrial metabolism, specifically through the depletion of the TCA cycle intermediate oxaloacetate. By integrating transcriptomic profiling of mitochondrial-incompetent NS1 mutants with metabolic rescue experiments, we have potentially established a causal link between mitochondrial dysfunction and the silencing of the type I and type III interferon responses. The convergence of transcriptional signatures between the NS1 mutants and cells supplemented with exogenous oxaloacetate provides compelling evidence for a shared mechanistic axis. The NS1 mutations Y125A and L132A/L133A disabled viral immune suppression, increasing expression of IFNB1, IFNLs, and CXCL11 [21–23]. Chemically restoring oxaloacetate in wild-type infection models phenocopied the genetic restoration of immunity. Wild-type NS1 protein in the mitochondria depletes local oxaloacetate, explaining this metabolic phenocopy [24]. The metabolite's absence prevents the cell from detecting a pathogen, blocking the bioenergetic shift needed for cytokine production.

Our machine learning analysis reveals that MDH1 and TBK1 are top predictors of the state of immunity, which explains this aspect at a molecular level. Cytosolic Malate Dehydrogenase 1 (MDH1) is a direct sensor of intracellular oxaloacetate levels [24–27]. In healthy or activated immune cells, high levels of oxaloacetate induce MDH1 dimer formation as a scaffold to facilitate the recruitment and phosphorylation of transcription factor ETS2. This complex transcribes the kinase TBK1, which phosphorylates IRF3 and

induces interferon. The RSV NS1 suppresses this axis at a node. By reducing mitochondrial oxaloacetate export, NS1 probably inhibits MDH1 dimerization effectively uncoupling metabolism sensing from TBK1 expression. At the same time, the model predicts that our mutant experiences a steady increase of TBK1 expression, which abolishes metabolic inhibition in our mutant and supplemented conditions. NS1 directly interferes with host signaling by means of STAT2 proteasomal degradation and sequestration of MAVS. An early, possibly primary, metabolic defect is associated with these described interactions. The immune response was only saved by providing oxaloacetate, indicating the influence of the metabolic checkpoint. It is possible that a lack of TCA cycle intermediates establishes a cellular context which promotes the degradation of STAT2 or destabilizes the MAVS signalosome. Metabolic collapse induced by NS1 potentiates its protein-level inhibition of host cells. Argument on NS1 intracellular localization focuses on how reduced function might explain it. Although the focus of our investigation has been on the mitochondrial function of NS1 with emphasis on Y125 and L132/133 playing an important role for the mitochondrial interface, published findings have also alluded to nuclear interactions with MED25 [28]. Such an activity is exerted by NS1 in two compartments with a redundancy of function. The mitochondrial checkpoint initially inhibits signaling by exhausting small molecule metabolites. Nuclear accumulation provides a secondary block, inhibiting residual transcription. The mutants used in our study likely disrupt the mitochondrial entry or function, thereby maintaining the oxaloacetate pool and allowing the MDH1-ETS2 signal to override the nuclear blockade. RSV must suppress host immune signaling to replicate.

Cross-validation with Influenza A datasets indicate respiratory viruses convergently evolved to sabotage host metabolism [29]. Influenza, Dengue, and Hepatitis B viruses alter mitochondrial dynamics or central carbon metabolism to support their replication [30]. Targeting the oxaloacetate-TBK1 axis by RSV NS1 is specific to the Pneumoviridae family. This specificity provides a rationale for the distinct clinical presentation of RSV, which often involves a protracted suppression of adaptive immunity and a failure to establish immunological memory, processes that are heavily dependent on the initial cytokine milieu established by the innate response [31]. Oxaloacetate limits antiviral immunity, presenting a therapeutic target for RSV, which lacks licensed host-directed antivirals. Metabolic rescue increases host cell resistance to viral infection. Unlike direct-acting antivirals that target viral proteins and drive the selection of resistant mutants, a metabolic therapy would target the immutable physiology of the host cell. Cell-permeable oxaloacetate analogs or MDH1-stabilizing small molecules provide a means to restore the interferon response during viral replication. This approach makes host cells inhospitable to the virus, limiting spread and lowering disease severity in susceptible populations.

Our findings linking NS1-mediated oxaloacetate depletion to immune suppression are based on in vitro human epithelial cell models and external dataset integration. Although we demonstrate that metabolic supplementation rescues the antiviral phenotype in these cells, we did not perform direct metabolomic profiling of mitochondrial versus cytosolic fractions to quantify the precise flux of oxaloacetate in situ. Furthermore, while the convergence of signatures between RSV mutants and Influenza models suggests a shared mechanism, the therapeutic efficacy of oxaloacetate supplementation has not yet been validated in vivo animal models of RSV infection. Validating these cellular findings in whole organisms will determine the clinical utility of metabolic rescue.

In summary, this report establishes RSV NS1 as a critical regulator of the immunometabolism interface. By tracing the transcriptomic footprint of NS1 mutants to a specific metabolic deficit, we have mapped a novel pathway of immune evasion that centers on the depletion of oxaloacetate. RSV suppresses broad innate immune responses by exploiting the system's reliance on metabolic signals. Future research should determine the structure of the NS1-mitochondrial interaction. This work can guide the clinical testing of metabolic rescue strategies that treat infection by restoring cellular function.

■ CONCLUSION

The RSV NS1 protein is a metabolic checkpoint inhibitor that suppresses innate immunity through mitochondrial oxaloacetate depletion. The metabolic blockade inhibits the MDH1-TBK1 signaling axis, preventing host cell detection of the virus. Transcriptional convergence between mitochondrial-incompetent NS1 mutants and oxaloacetate-supplemented cells shows that restoring metabolic homeostasis restores antiviral defenses. This mechanism suggests a therapeutic approach for respiratory viral infections.

■ REFERENCES

1. Gatt D, Martin I, AlFouzan R, Moraes TJ. Prevention and Treatment Strategies for Respiratory Syncytial Virus (RSV). *Pathogens*. 2023 Jan 17;12(2):154.
2. Asseri AA. Respiratory Syncytial Virus: A Narrative Review of Updates and Recent Advances in Epidemiology, Pathogenesis, Diagnosis, Management and Prevention. *J Clin Med.* 2025 May 30;14(11):3880.
3. Loe MWC, Soenong H, Lee E, et al. Nirsevimab: Alleviating the burden of RSV morbidity in young children. *J Paediatr Child Health.* 2024;60(10):489–98.
4. Wilkins D, Wählby Hamrén U, Chang Y, et al. RSV Neutralizing Antibodies Following Nirsevimab and Palivizumab Dosing. *Pediatrics.* 2024 Oct 1;154(5):e2024067174.
5. D'Ambrosio F, Lomazzi M, Moore M, et al. Addressing the Underestimated Burden of RSV in Older Adults in Europe: Epidemiology, Surveillance Gaps, and Public Health Implications. *Vaccines.* 2025 May 12;13(5):510.
6. Kim T, Choi SH. Epidemiology and Disease Burden of Respiratory Syncytial Virus Infection in Adults. *Infect Chemother.* 2024 Mar;56(1):1–12.
7. Hanage WP, Schaffner W. Burden of Acute Respiratory Infections Caused by Influenza Virus, Respiratory Syncytial Virus, and SARS-CoV-2 with Consideration of Older Adults: A Narrative Review. *Infect Dis Ther.* 2025 Jan 1;14(1):5–37.
8. Morens DM, Taubenberger JK, Fauci AS. Rethinking next-generation vaccines for coronaviruses, influenzaviruses, and other respiratory viruses. *Cell Host Microbe.* 2023 Jan 11;31(1):146–57.
9. Georgakopoulou VE, Pitiriga VC. Immunomodulation in Respiratory Syncytial Virus Infection: Mechanisms, Therapeutic Targets, and Clinical Implications. *Microorganisms.* 2025 Aug 12;13(8):1876.
10. Royen TV, Rossey I, Sedeyn K, et al. How RSV Proteins Join Forces to Overcome the Host Innate Immune Response. *Viruses*. 2022 Feb 17;14(2):419.
11. Merritt TN, Pei J, Leung DW. Pathogenicity and virulence of human respiratory syncytial virus: Multifunctional nonstructural proteins NS1 and NS2. *Virulence*. 2024 Dec 31;15(1):2283897.
12. Yang Q, Elz AE, Panis M, et al. Modulation of Influenza A virus NS1 expression reveals prioritization of host response antagonism at single-cell resolution. *Front Microbiol.* 2023 Oct 9;14:1267078.
13. Li J, Wang Y, Deng H, et al. Cellular metabolism hijacked by viruses for immunoevasion: potential antiviral targets. *Front Immunol.* 2023 July 25;14:1228811.
14. Kanno T, Miyako K, Endo Y. The diverse interaction of metabolism, immune response, and viral pathogens. *Front Immunol.* 2025 July 29;16:1619926.
15. Safadi DE, Paulo-Ramos A, Hoareau M, et al. The Influence of Metabolism on Immune Response: A Journey to Understand Immunometabolism in the Context of Viral Infection. *Viruses.* 2023 Dec 9;15(12):2399.
16. Palmer CS. Innate metabolic responses against viral infections. *Nat Metab.* 2022 Oct;4(10):1245–59.
17. O'Carroll SM, Henkel FDR, O'Neill LAJ. Metabolic regulation of type I interferon production. *Immunol Rev.* 2024;323(1):276–87.
18. Camps J, Iftimie S, Jiménez-Franco A, Castro A, Joven J. Metabolic Reprogramming in Respiratory Viral Infections: A Focus on SARS-CoV-2, Influenza, and Respiratory Syncytial Virus. *Biomolecules.* 2025 July 16;15(7):1027.
19. Martín-Vicente M, González-Riaño C, Barbas C, et al. Metabolic changes during respiratory syncytial virus infection of epithelial cells. *PLoS ONE.* 2020 Mar 26;15(3):e0230844.
20. Li D, Zand MS, Dye TD, et al. An evaluation of RNA-seq differential analysis methods. *PLoS ONE*. 2022;17(9):e0264246.
21. Kalita P, Khatavkar O, Uwase G, et al. Molecular basis for human respiratory syncytial virus transcriptional regulator NS1 interactions with MED25. *Nat Commun.* 2025 Mar 25;16:2883.
22. Ait-Mouhoub C, Dong J, Noiray M, et al. A dual interaction between RSV NS1 and MED25 ACID domain reshapes antiviral responses. *PLOS Pathog.* 2025 Sept 8;21(9):e1012930.
23. Man SM, Singh N, Sercus J, et al. Nonstructural protein 1 of human respiratory syncytial virus promotes HMGB1 release and airway inflammation by targeting histone H1.0. *Inflammation.* 2025 May 14;48:3579–91.

24. Zúñiga S, Patel MC, Pearson JA, et al. Multifaceted mitochondria in innate immunity. *NPJ Metab Health Dis.* 2024;4(1):15.
25. Jin S, He X, Wang Z, et al. Oxaloacetate sensing promotes innate immune antiviral defence against influenza virus infection. *Nat Microbiol.* 2025 Oct;10(10):2521–36.
26. McCue WM, Finzel BC. Structural characterization of the human cytosolic malate dehydrogenase I. *ACS Omega.* 2022;7(1):207–14.
27. Broeks MH, Shamseldin HE, Alhashem A, et al. MDH1 deficiency is a metabolic disorder of the malate–aspartate shuttle associated with early onset severe encephalopathy. *Hum Genet*. 2019 Dec 1;138(11):1247–57.
28. Van Royen T, Sedeyn K, Moschonas GD, et al. An Unexpected Encounter: Respiratory Syncytial Virus Nonstructural Protein 1 Interacts with Mediator Subunit MED25. *J Virol.* 2022 Oct 12;96(19):e01297–22.
29. Girdhar K, Powis A, Raisingani A, et al. Viruses and Metabolism: The Effects of Viral Infections and Viral Insulins on Host Metabolism. *Annu Rev Virol.* 2021 Sept 29;8:373–91.
30. Duan X, Liu R, Lan W, Liu S. The Essential Role of Mitochondrial Dynamics in Viral Infections. *Int J Mol Sci.* 2025 Feb 24;26(5):1955.
31. Nouari W, Aribi M. Innate lymphoid cells, immune functional dynamics, epithelial parallels, and therapeutic frontiers in infections. *Int Rev Immunol.* 2025 Apr 17;44(5):245–72.

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An Epidemiological and Physiological Study of Head Lice Infestation among Primary School Students

Conflict of interest: nothing to declare.
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Abstract

Introduction. Head lice (Pediculus humanus capitis) is a global health problem, and it is more prevalent among primary school children due to its presence on the hair and scalp. Infestation is transmitted through physical contact and sharing combs, towels, and clothing.

Purpose. To determine the prevalence of head lice according to sex, age, number of household members, and season, as well as to investigate the effect of this parasite on blood parameters in participating primary school students.

Materials and methods. This study was conducted in Al-Rifai District, Thi-Qar province, and included 385 primary school students (105 males and 280 females) between October 2025 and March 2026, A comprehensive examination was performed to detect infections using visual inspection and accurate diagnosis, Samples were then collected and analyzed, and blood samples were drawn for laboratory testing. A questionnaire on the study variables was also completed.

Results. The prevalence of head lice among males and females was 12.38% and 26.42%, respectively. A significant increase was observed among children aged 9–10 years (33.59%), while a significant decrease (16.28%) was recorded among children aged 7–8 years. The highest prevalence was recorded in the fourth grade (35.38%), while the lowest was in the sixth grade (7.57%). The highest infestation rate was observed among students whose families consisted of more than seven members. The months of the year also had a clear impact on the occurrence of infestation. The highest rates of head lice infestation were observed during December, January, and November 26.82%, 25.97%, and 22.05%) respectively, with a clear decrease during February and March 17.30% and 18.18%, respectively. The study results also indicated a significant increase in the white blood cell count and a decrease in total red blood cell count, hemoglobin and HCT at a probability level ($P<0.05$).

Conclusion. The direct contact between children in the school environment is a major cause of head lice infestation. It is less common among boys than girls and more prevalent among students living in large families with more than seven members. Infestations are most common during the winter and autumn months. Therefore, it is crucial to raise awareness and provide information on how to detect, control, and eliminate head lice, as it can affect blood parameters and, in some cases, lead to severe anemia along with other side effects.

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Keywords: epidemiological study, physiological study, head lice, pediculus humanus capitis

■ INTRODUCTION

Head lice (Pediculus humanus capitis) is a common health problem affecting people of all ages, impacting millions of children worldwide [1]. Studies have shown considerable variation in infestation rates, reaching up to approximately 80% [2]. This poses a significant health concern, causing anxiety, distress, and embarrassment for parents [3]. Head lice are external parasites belonging to the arthropod family, ranging in length from 1 to 3 mm, they have short antennae, sucking mouthparts, and three pairs of legs [4]. They are most prevalent among students aged 3 to 11 years and between 24 and 36 years. Individuals in close contact with infected persons are also at risk of infestation [5], Lice live on the scalp, where they feed about 4–5 times a day by injecting saliva and sucking blood directly. Their saliva has vasodilators and anticoagulant properties. A female louse lays about 150 eggs during her life cycle. Nits are more easily seen than adult lice at the back of the head and behind the ears. Nits can survive for up to ten days, while adult lice survive for about three days away from the host [6]. The spread of lice depends on weather conditions, with infestations being more common in winter [7]. In addition, several factors influence the spread of lice, including age, sex, hair characteristics, parental education level, and social and economic standing [8].

The most important symptoms of head lice infestation are anemia, severe irritation, and itching of the scalp due to saliva and fecal antigens that cause an allergic reaction in the host [6]. Sometimes, some children do not show symptoms for one to two months during the initial infestation period because the allergy is delayed in responding to the parasite's antigens. Therefore, a group of early symptoms such as fatigue, irritability, and lethargy appear in the patient, and then the head lice infestation is confirmed [3]. Direct contact between infected individuals is considered one of the most common ways for the infection to spread and occur [8]. Head lice depend on human blood for survival and reproduction. They feed by directly biting the capillaries in the host's scalp. Although absorption and effect seem unclear, the loss of small amounts of host blood on a regular basis lead to iron deficiency and anemia during continuous feeding [9].

The main objective of this study is to determine the extent of lice infestation in Al-Rifai district within Thi-Qar Province and to know the effect of other factors such as age group, gender, educational stages and seasonal spread of head lice on the occurrence of infection among primary school students. In addition, the scientific references do not contain data on the effect of lice on complete blood parameters and on human health during the period of infestation, so this study was conducted to find out if this is of clinical importance.

■ MATERIALS AND METHODS

This study was conducted at the beginning of the academic year, from October 2025 to March 2026, Samples were collected from 385 students (105 males and 280 females) aged 7 to 12 years from Al-Rifai district in Dhi Qar Governorate. The study included primary school students from two schools within the lowest socioeconomic stratum

of the study population. A complete census of all children was conducted, and they underwent lice examination at school, through careful visual inspection and the use of a fine-toothed comb to check the back of the neck and behind the ears for infestation [6, 11]. Subsequently, lice samples were collected and examined under a microscope in the life sciences laboratory. Blood samples (in milliliters) were then drawn from infested individuals (5 males and 5 females) and uninfected individuals (5 males and 5 females) and placed in tubes containing anticoagulant (EDT) for a complete blood count (CBC). Data on age, sex, school year, month of year, and number of household members were recorded.

Statistical analysis

These were then analyzed using SPSS software and the chi-squared test to compare variables. The least significant difference (LSD) was used at a probability level of 0.05. Blood parameters were analyzed using the CRD randomization method in all statistical analyses of head lice prevalence [12].

■ RESULTS

The current study included a sample of 385 primary school students. The highest prevalence of head lice was recorded among females (74, 26.42%) compared to males (13, 12.38%), with a statistically significant difference ($P<0.005$), as shown in Table 1. A statistically significant increase in the prevalence was observed among the 9–10 year age group (33.59%), while a statistically significant decrease was observed among the 7–8 year age group (16.28%) (Table 2). The highest statistically significant differences were recorded between third-grade students (31.74%) and fourth-grade students (35.38%), followed by the lowest prevalence among sixth-grade students (7.57%) (Table 3). No statistically significant differences were observed based on family size. The highest percentages 24.24% and 23.38% were recorded among families of seven or more members and among families of 5–6 members, and 15.78% among families of 2–4 members (Table 1). As for the months of the year, the highest percentages (26.82%) were observed in December, while the lowest percentages 17.30% and 18.18% were recorded in February and March, respectively, without any statistically significant difference ($P>0.05$), as shown in Table 5.

The study results indicated a significant decrease in red blood cell count among affected males (2.84±0.20), while the decrease was less pronounced among affected females (2.44±0.29). A significant decrease in hemoglobin levels was also recorded, with the lowest levels observed in females (11.45±0.30) compared to males (12.45±0.40) when compared to the control group for both sexes, a significant decrease in hematocrit (HCT) was observed in infected males (38.92±0.42) compared to the control group (42.61±0.28), followed by a similarly significant decrease in infected females (35.74±0.32). The infection also led to a significant increase in white blood cell count in infected females (9.53±0.24), which was higher than in the control group. A similarly significant increase was observed in infected males (8.98±0.26) at a statistically significant level ($P<0.05$), as shown in Table 6.

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Table 1
Lice infestation by gender among students

Sex	Non-infected (%)	Infected (%)	Total
Males	92 (23.89)	13 (12.38)	105 (27.27)
Females	206 (53.51)	74 (26.42)	280 (72.72)
	P = 0.005 Chi-Shuare = 7.831		

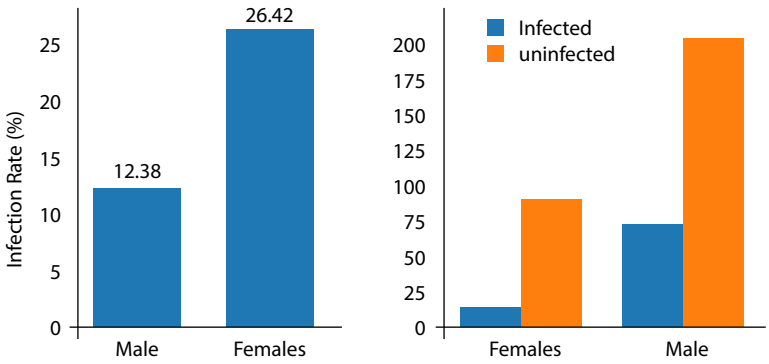

Fig. 1. Shows the percentage of lice infestation by sex

Table 2
Lice infestation according to the age group of students

Age group	Examined samples	Infected samples	Uninfected samples	Infection (%)
7–8	130	21	109	16.28
9–10	132	43	89	33.59
11–12	123	23	100	17.96
Total	385	87	298	22.59
	P=0.0029 Chi-Shuare = 11.669			

Table 3
Lice infestation according to the educational stage of the students

Academic stage	Examined samples	Infected samples	Uninfected samples	Infection (%)
First grade	67	9	58	13.43
Second grade	62	12	50	19.35
Third grade	63	20	43	31.74
Fourth grade	65	23	42	35.38
Fifth grade	62	18	44	29.03
Sixth grade	66	5	61	7.57
	p=0.0004 Chi-Shuare = 22.663			

Table 4
Lice infestation according to family size for students

Family size	Examined samples	Infected samples	Uninfected samples	Infection (%)
2–4	63	01	53	15.78
5–6	124	29	95	23.38
≥7	198	84	150	24.24
	P=0.3717 Chi-Shuare=1.979			

Table 5
Lice infestation according to the months of the year

Months	Examined samples	Infected samples	Uninfected samples	Infection (%)
October	62	13	49	20.96
November	68	15	53	22.05
December	82	22	60	26.82
January	77	20	57	25.97
February	52	9	43	17.30
March	44	8	36	18.18
	P=0.7355 Chi-Shuare=2.769			

Table 6
Blood parameters among students infected with head lice

Groups	R.B.C (μL/×10⁶)	Hb (dL/g)	H.C.T (%)	W.B.C (Lμ/×10³)
Males				
Uninfected group	4.99±0.03 a	14.65±0.58 a	42.61±0.28 a	6.72±0.35 d
Infected group	2.84±0.20 c	12.45±0.40 c	38.92±0.42 b	8.98±0.26 b
Females				
Uninfected group	4.70±0.23 b	13.48±0.27 b	38.48±0.28 c	7.23±0.16 c
Infected group	2.44±0.29 d	11.45±0.30 d	35.74±0.32 d	9.53±0.24 a
LSD	0.19	0.37	0.30	0.24

Notes: * Standard deviation ± average. * Different letters vertically indicate a significant difference between the groups at a probability level (P<0.05).

■ DISCUSSION

Despite advancements in health and medical awareness, it remains a significant health problem in poor and developing countries [8]. As the first study to address this widespread problem in Al-Rifai District, the representing a prevalence rate of 22.59%. The infested individuals were found to be infested with immature lice, adult lice, or nits (lice eggs).

Our study is consistent with a number of studies, as it reached 13.5% among primary school students in Baghdad [13], and in Mosul it was found that 33.2% of children were infected with lice [14], while in Najaf the infection rate was 14% [15], while a lower prevalence of head lice was recorded among primary school children in Nasiriyah Governorate at 7.2% [14]. Thus, the infection rate varies considerably between different countries [8]. It was noted in the current study that the prevalence of head lice among male school students (12.38%) was lower than its prevalence among females (26.42%).

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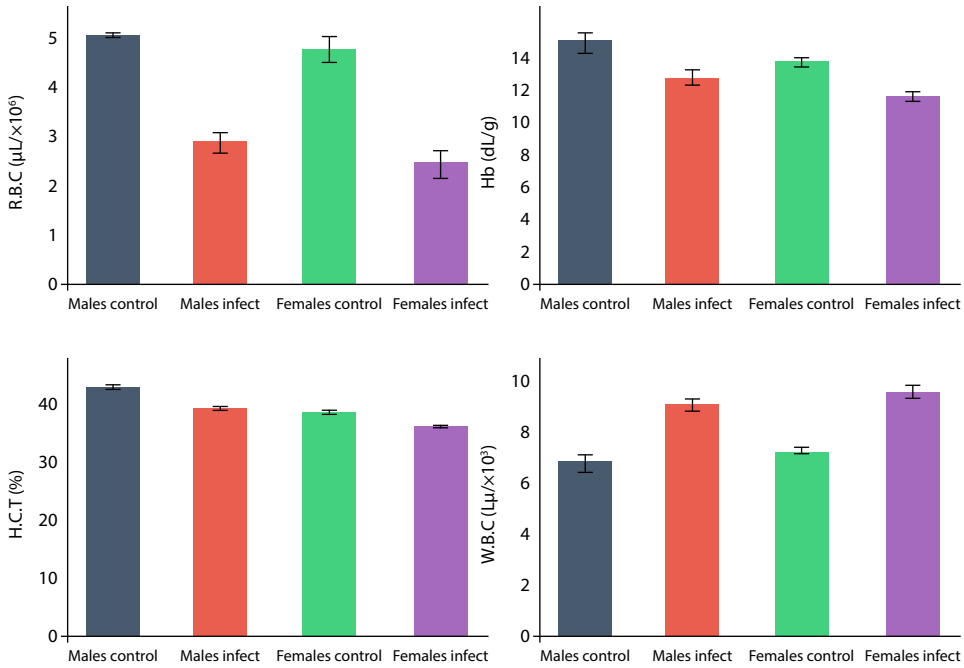

Fig. 2. Illustrates the changes in blood parameters when infected with head lice

The reason for this difference in the prevalence of head lice infection may be attributed to several factors, including the length of girls' hair, which hinders the immediate detection of the infection, as the eggs remain attached with the hair growth, thus causing repeated infections [16]. Compared to shorter male hair, there are also differences in behaviors and circumstances, such as direct head-to-head contact between males and females during sports and daily play. Females also tend to have more direct and prolonged contact, and lice can be transmitted via personal hygiene items such as hairbrushes and towels [17]. This is consistent with a study [18], and another study found that the prevalence of head lice among females (38.66%) was higher than among males (6.27%) [8]. This is consistent with a study conducted in Benghazi, where a rate of 88.1% was recorded among females and 67.2% among males [19]. In Iran, the prevalence of lice was 2.383% among female students, while it was 0.11% among male students [20]. This is also consistent with a study that found the prevalence of head lice to be higher among girls [21]. The researcher [22], in his study of the prevalence of head lice among primary school children in Kenya, concluded that infestation was not related to sex [23], also found that the distribution of head lice between females and males was very similar. Our results do not agree with those of a study conducted on students, which showed the highest infestation rate, at 2.74% among females and 3.39% among males [17].

The results showed that the 9–10 year age group had the highest infestation rate (33.59%), followed by the 11–12 year age group, compared to the 7–8 year age group.

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Our study is consistent with a study [13] conducted in Baghdad, which found that head lice prevalence is significantly influenced by age, with a remarkably higher rate (18.7%) recorded in the 8–10 year age group compared to other age groups. This is attributed to the fact that younger children receive care and supervision from their mothers or older siblings, allowing them greater independence. In addition, girls tend to have longer hair and are more social, increasing the likelihood of direct heads contact. Close contact with friends and family affects the infestation rate. Furthermore, the Iraqi education system's reliance on double desks increases physical contact among primary school children, allowing for the transmission of lice from person to person and creating a suitable environment for its spread. These factors, along with others, may explain why this age group is more susceptible to head lice. Our study is consistent with [24], which found the highest infestation rate among children aged 6–12 (9.7%), with the peak at age 9. A study [6] conducted on girls aged 7–9 found them more susceptible to lice than girls aged 10–12. Another study [11] showed the highest infestation rate in the 8–9 age group, while the lowest rate was among children aged 12 and older. Our findings are consistent with those of study [25]. A study conducted in Jordan indicated a high rate of head lice infestation among younger children [26]. Contrary to previous studies [6], the highest rate of head lice infestation was found in the 10–11 age group, compared to the 6–7 and 8–9 age groups. This aligns with the findings of the study [27]. This contradicts the results of several previous studies, such as the study [28], which confirmed that the highest rate of infestation was among elementary school children aged six.

Our study found a strong correlation between the prevalence of head lice and school stage. There was a high incidence of head lice in the third and fourth grades, at 31.74% and 35.38% respectively, compared to other school stages. Our results are similar to the results of study [29], This can be explained by the fact that younger children are often under the care of their parents regarding hair cleaning, combing, and styling, allowing for early detection and quick treatment of infestations. In contrast, older children often rely on themselves for cleaning and combing their hair, and their mothers may not be as attentive to their personal hygiene. This aligns with a study [30] which found the highest rate of head lice infestation in the fifth grade, i.e., at age eleven, compared to other age groups [31], reported that the infestation rate was higher among first-grade students (5, 19). The infestation rate also increases in families with more than three members, as our study found. This is attributed to the sharing of hairbrushes, hats, clothes, and pillows among family members, coupled with poor personal hygiene practices and a lack of concern about head lice infestations. Thus, the larger the family size, the less parental attention is given to the children's health, and the closer contact between individuals in crowded families leads to a higher rate of transmission and an increased risk of infection from siblings [32], also found that one of the contributing factors to the spread of infestation is the number of children in the family. Family and the level of healthcare at home and school, and this is consistent with a study [33]. This is consistent with study [31], which found that the highest infection rate 60% was observed in a family consisting of 12 individuals. In contrast, study [8] found that most of those infected with head lice belong to families consisting of 5–6 individuals or more.

Our study results showed that infections reached their peak in December, January and November and decreased during February and March, as head lice usually spread in winter and in cold and temperate climates [34]. This is consistent with a study [15] that showed

that the season has a major impact on the spread of head lice. It found that students in winter and autumn are more susceptible to head lice compared to spring. The highest infection rate was recorded in January (19.91%), while the lowest rate was recorded in April (11.60%). This increase in the rate of head lice infestation is likely explained by the cold weather, which makes children wear warm clothes and hats, often sharing them with their siblings and classmates or leaving them in the classroom, thus increasing the chances of transmission. In addition, there is increased crowding in classrooms during play and rest, thus increasing physical contact between children. This direct contact decreases during March and April when the weather becomes less cold, which in turn gives children more freedom to play with less contact between them. The results of our study are similar to previous studies [7]. Moreover, a study [35] conducted in Iran reported an increase in the prevalence of head lice in the autumn and winter seasons with a decrease in infestation in the spring, while a study [36] conducted on primary school students in Egypt reported an increase in the prevalence of head lice in the humid climate and in the summer. Thus, there is a relationship between seasonal variation and the prevalence of head lice, as climate change affects the lifespan of head lice, their ability to reproduce, their transmission, and the occurrence of infection [7].

In our current study, a decrease in the red blood cell count, hemoglobin, and HCT was observed, along with a clear increase in the white blood cell count. This is consistent with a study [15] that showed that the incidence of anemia reached (9.80%) among children suffering from head lice infestation. This is attributed to head lice feeding on human blood, which leads to iron absorption and an imbalance, causing a severe iron deficiency in cases of severe infestation due to head lice consuming blood more than three times a day. This causes fatigue and drowsiness during school classes and learning difficulties. Chronic infestation among school children may lead to anemia in all of them [37], in addition to the occurrence of common complications due to bacterial infection at the bite site, especially during repeated infestation [38]. Since elementary school students are more susceptible to head lice infestation, incorrect behavioral patterns and continuous scratching at the bite site lead to the impact on blood parameters and the occurrence of secondary bacterial infections [39].

■ CONCLUSIONS

Head lice are a major health problem in many developed countries. According to our study, the rate of lice infestation appears to be higher among females than males, especially those who are self-reliant in hygiene and personal care in crowded households. This can affect the general health of those infected, leading to anemia and bacterial infections during both cold and warm seasons.

■ REFERENCES

1. Khidhir KN, Mahmood CK, Ali WK. Prevalence of infestation with head lice Pediculus humanus capitis (De Geer) in primary schoolchildren in the centre of Erbil City, Kurdistan. *Pakistan Entomologist*. 2017;39(2):1–4.
2. Mohamed K, Mubarak AAL, Zaghloul D, et al. Prevalence of Head Lice (Pediculus humanus capitis) Infestation among Pupils in Elementary Schools in Makkah, Saudi Arabia. *International Journal of Medical Research & Health Sciences*. 7(8):66-76
3. Nutanson I, Steen CJ, Schwartz RA, Janniger CK. Pediculus humanus capitis: an update. *Acta Dermatovenerol Alp Pannonica Adriat.* 2008 Dec;17(4):147-54, 156-7, 159. PMID: 19104739.
4. Meister L, Ochsendorf F. Head Lice. *Dtsch Arztebl Int.* 2016;113(45):763–773.

5. Bekri G, Shaghaghi A. Prevalence of Pediculus humanus capitis and associated risk factors among elementary school-aged girls in Paveh, West Iran. *J Infect Dev Ctries.* 2022.
6. Amin OM, Mahmood HFD, Muhammed AA, Sawsan A. Prevalence of head lice, Pediculus *humanus capitis* L. and their relation to anxiety among primary school children in Kalar district, Kurdistan region-Iraq. *Journal of University of Garmian.* 6 (2), 2019. https://doi.org/10.24271/garmian.196226
7. Ziaoddini AA, Riahi R, Heidari-Beni M, et al. National and Provincial Prevalence of Pediculus humanus capitis among Urban Students in Iran from 2014 to 2018. *J homepage.* 2019;19(4):4–8.
8. Saghafipour A, Nejati J, Zahraei-Ramazani A, Vatandoost H. Prevalence and Risk Factors Associated with Head Louse (Pediculus humanus capitis) in Central Iran. *Int J Pediatr.* 2017;5(43):5245–5254.
9. Speare R, Canyon DV, Melrose W. Quantification of blood intake of the head louse: Pediculus humanus capitis. *Int J Dermatol.* 2006 May;45(5):543-6. doi: 10.1111/j.1365-4632.2005.02520.x. PMID: 16700788.
10. Matrood A, Almaeahi SAA. Study diagnostic for lice parasite with Some haematological changes in Passer domesticus in Al-Qadisiya province. *Al-Qadisiyah Univ.* 2013.
11. Al-Daoody AAK, Mohammed AK, Jabbary HHM, Ali FA, Ibrahim HA, Abdullah KM, Mawlud SI, Rahman SJ. Investigation on prevalence, risk factors, and genetic diversity of Pediculus humanus capitis among primary school children. *Cell Mol Biol (Noisy-le-grand).* 2022 Jan 2;67(4):382-389. doi: 10.14715/cmb/2021.67.4.44. PMID: 35809265.
12. Zubairi RB, Hasan SA, Okab HF. Effect of IL-17 and IL-23 Levels in Women with Polycystic Ovary Syndrome. *Reprod Heal East Eur.* 2025;15(1):43–50.
13. Mahmood SA. Head pediculosis among in Baghdad area elementary schoolchildren *Pediculus humanus capitis*. *Iraqi Journal of Science.* 2010;51(1):49–55.
14. Al-Abaddy I. Investigation on the incidence of infection of lice among human goats and sheep in Mosul city-Iraq. 2008:1–7.
15. Khidhir WK, Mahmood CK, Ali WK. Prevalence of infestation with head lice Pediculus humanus capitis (De Geer) in primary schoolchildren in the centre of Erbil city, Kurdistan region, Iraq. *Pak Entomol.* 2017;39(2):1–4.
16. Bahrami Abbas AE, Nikfallah F, Soleimanfallah M, et al. Clinical and epidemiological study of pediculosis, Pediculus humanus capitis (head lice infestation) in primary school pupils, Savojbolagh County, Alborz Province, Iran. *Eur J Biomed Pharm Sci.* 2016;3(3):60–64.
17. Abd H, Zaed A. Infestation of Head Lice, Pediculus humanus capitis, in Primary School Children at Houn City, Libya. *Journal of Academic Research*. 2019;(13):38–52.
18. Lesshafft H, Baier A, Guerra H, Terashima A. Prevalence and Risk Factors Associated with Pediculosis Capitis in an Impoverished Urban Community in Lima, Peru. *J Glob Infect Dis.* 2013;4.
19. Bharija BM, Kanwar AJ. Pediculosis Capitis in Benghazi, Libya. *Int J Dermatol.* 1988;27:165–166.
20. Omidi A, Khodaveisi M, Moghimbeigi A, Mohammadi N, Amini R. Pediculosis capitis and relevant factors in secondary school students of Hamadan, west of Iran. *J Res Health Sci.* 2013 Sep 17;13(2):176-80. PMID: 24077476.
21. Oguzkaya Artan M, Baykan Z. The prevalence of Pediculus capitis in eight primary schools of Kayseri (in Turkish). *Turkiye Parazitol Derg.* 2006;30:112–114.
22. Chunge RN. A study of head lice among primary schoolchildren in Kenya. *Trans R Soc Trop Med Hyg.* 1986;80(1):42-6. doi: 10.1016/0035-9203(86)90191-4. PMID: 3726995.
23. Boyle P. Pilot study of the prevalence of head lice infestation in a population of Saudi Arabian children. *Fam Pract.* 1987 Jun;4(2):138-42. doi: 10.1093/fampra/4.2.138. PMID: 3609553.
24. Değerli S, Malatyali E, Çeliksöz A, et al. The Prevalence of Pediculus humanus capitis and the Coexistence of Intestinal Parasites in Young Children in Boarding Schools in Sivas, Turkey. *Pediatr Dermatol.* 2012;29(4):426–429.
25. Kalari, H., Soltani, A., Azizi, K. *et al.* Comparative efficacy of three pediculicides to treat head lice infestation in primary school girls: a randomised controlled assessor blind trial in rural Iran. *BMC Dermatol* **19**, 13 (2019). https://doi.org/10.1186/s12895-019-0093-5
26. Mohammed AL. Head lice infestation in schoolchildren and related factors in Mafraq governorate, Jordan. *Int J Dermatol.* 2012 Feb;51(2):168-72. doi: 10.1111/j.1365-4632.2011.04972.x. PMID: 22250625.
27. El-sayed MM, Toama MA, Abdelshafy AS, et al. Prevalence of pediculosis capitis among primary school students at Sharkia Governorate by using dermoscopy. *Egypt J Dermatol Venereol.* 2017:33–42.
28. Soliman EF. Prevalence of Pediculosis among Urban–Rural School Children in Misurata–Libya. *Third Symp Theor Appl Basic Biosci.* 2016 Sep 3.
29. J Alempour Salemi, N Shayeghi, H Zeraati, K Akbarzadeh, H Basseri, B Ebrahimi, J Rafinejad. Some Aspects of Head Lice Infestation in Iranshahr Area (Southeast of Iran). *Iran J Public Health*. 1;32(3):60-63.
30. Ghofleh Maramazi H, Sharififard M, Jahanifard E, Maraghi E, Mahmoodi Sourestani M, Saki Malehi A, Rasaei S. Pediculosis humanus capitis Prevalence as a Health Problem in Girl's Elementary Schools, Southwest of Iran (2017-2018). *J Res Health Sci.* 2019 Jun 17;19(2):e00446. PMID: 31278215; PMCID: PMC7183542.
31. Shayeghi M, Paksa A, Salim Abadi Y, et al. Epidemiology of Head Lice Infestation in Primary School Pupils, in Khajeh City, East Azerbaijan Province, Iran. *Iran J Arthropod-Borne Dis.* 2010;4(1):42–46.
32. Al-zayyadi SW. Study of prevalence of Pediculus humanus capitis among primary school pupils in Al-Najaf Al-Ashraf Province. *Biochem. Cell. Arch.* 2018;18(1950):1141–1143.
33. Van der Wouden JC, Klootwijk T, Le Cleach L, et al. Interventions for treating head lice (Protocol). *Cochrane Database Syst Rev.* 2018;(5):2–5.
34. Jackson DG. Hyaluronan in the lymphatics: The key role of the hyaluronan receptor LYVE-1 in leucocyte trafficking. *Matrix Biol.* 2019;78–79:219–235.
35. Vahabi A, Shemshad K, Sayyadi M, et al. Prevalence and risk factors of Pediculus (humanus) capitis (Anoplura: Pediculidae), in primary schools in Sanandaj City, Kurdistan Province, Iran. *Trop Biomed.* 2012;29(2):207–211.
36. Farag Mostafa F, Abdel A, Hassan H, et al. Prevalence of Skin Diseases among Infants and Children in Al Sharqia Governorate, Egypt. *Egypt J Dermatol.* 2012;8(1):1–14.
37. Guss DA, Koenig M, Castillo EM. Original Contributions. *J Emerg Med.* 2011;41(4):362–365.
38. Mazurek NP, Lee CM. How to manage head lice. *West J Med.* 2000;172:342–345.
39. Yousefi S, Shamsipoor F, Abadi YS. Epidemiological Study of Head Louse (Pediculus humanus capitis) Infestation Among Primary School Students in Rural Areas of Sirjan County, South of Iran. *Thrita.* 2012;1(2):53–56.

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Immunological Assessment Response in Ocular Infectious Illness with Staphylococcus Aureus

Conflict of interest: nothing to declare.
Authors' contribution: Zainab A. Ali – conceptualization, data curation, investigations, methods, project administration, resources, software, validation, visualization, writing – original draft and writing – review & editing; Huda Q. Tehymesh – conceptualization, data curation, methods, resources, supervisor, writing – original draft and writing – review & editing.
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Abstract

Introduction. Staphylococcus aureus is an opportunistic pathogen responsible for a variety of systemic and ocular infectious illnesses.
Purpose. This work investigated the prevalence of S. aureus in external ocular infectious illnesses, determined selected virulence genes, and evaluated linked immunological responses via measuring serum interleukin-6 (IL-6) and interleukin-8 (IL-8).
Materials and methods. A total of 213 ocular specimens were collected from patients attending Ibn Al-Haitham Hospital and private clinics in Baghdad among 3 September 2023 and 3 March 2024. Bacterial identification was performed applying culture characteristics, microscopic examination, biochemical tests, and confirmation with the API-Staph system. Virulence genes were detected via molecular methods. Serum IL-8 and IL-6 levels were measured and compared with healthy controls applying statistical analysis.
Results. Bacterial growth was observed in 122 (57.27%) samples. Gram-positive isolates constituted 94 (77.04%), whereas Gram-negative isolates accounted for 28 (22.95%). S. aureus was the predominant pathogen, representing 54/122 (40.9%). Clinically, conjunctivitis was the most common presentation (36%), followed via blepharoconjunctivitis (24%), blepharitis (16%), dacryocystitis (13%), and other external ocular infectious illnesses (5%). The prevalence of virulence genes was 94.5% for sea, 39.68% for seb, 34.92% for sec, and 28.57% for sed. Patients infected with S. aureus showed significantly higher serum IL-6 (83.89±1.68 pg/mL) and IL-8 (206.81± 16.61 pg/mL) compared with controls (67.82±4.28 pg/mL and 78.45±6.36 pg/mL; $P<0.05$).
Conclusions. S. aureus is a leading cause of external ocular infectious illnesses and is linked with increased expression of virulence determinants and elevated inflammatory cytokines, underscoring its clinical importance in ocular pathology.
Keywords: IL-6, IL-8, eye infectious illness, S. aureus, sea gene

■ INTRODUCTION

The eye is a highly specialized body organ that is always in contact with its environment and hence have a critical function as an interface among the body and external factors.

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As such, infectious illnesses in the eye, especially those caused via microbial pathogens, are a major public health problem worldwide, with a higher prevalence being noted in poor and disadvantaged regions [1]. Infectious conditions involving the eye may compromise the integrity of ocular tissues and, when not accurately diagnosed or adequately managed, can result in visual impairment or even permanent blindness. Microbial involvement most commonly affects the corneal surface, eyelids, and conjunctiva, which are particularly vulnerable to pathogen colonization and subsequent inflammatory damage. S. aureus is an opportunist that infects humans' bodies, causing both ocular and systemic infectious illnesses. Over the course of the day, this bacterium becomes more resistant to various antibiotic classes.

S. aureus produces many virulence factors that trigger inflammatory reactions and tissue damage, as well as different cytokines, such as interleukin-6 (IL-6) [2]. Although it is mostly a proinflammatory cytokine, IL-6 can also function as an anti-inflammatory cytokine via inhibiting TNF-α, and it is implicated in both inflammation and homeostatic processes. It is IL-6 that have a pivotal function in T cell recruitment and B and T cell synthesis during inflammation and delayed T cell death [3]. Furthermore, IL-8 have a function in attracting T cells, Basophils, and Neutrophils [4]. Research has demonstrated that TNF-α triggers the production of IL-8, whereas IL-10 suppresses its production. IL-10 is a cytokine that is anti-inflammatory that have a vital function in controlling immunological responses. IL-10 inhibits the production of proinflammatory cytokines like IL-6 and decreases the expression of Major histocompatibility complex (MHC) class II molecules, T helper type 1 cytokines and TNF-α [5].

■ MATERIALS AND METHODS

Collection of samples

A total of 213 clinical specimens were collected from patients who presented symptoms of ocular infectious illnesses at the ophthalmic consultation clinic of Ibn Al-Haitham Hospital in Baghdad. The samples were collected from three anatomical sites of the eye, including the eyelids, cornea, and conjunctiva. The work was conducted among April and August 2024. Ethical and administrative clearance to carry out the research was approved via the Baghdad Health Directorate. The participants of both sexes were chosen applying simple random sampling. The number of samples was computed applying an established statistical formula [6]:

$$\frac{Z_{1-\alpha/2^2}p(1-p)}{d^2}.$$

Applying the level of significance of 5% (p=0.05), the $Z_{1-\alpha/2}$ value of A standard ordinary variate was 1.96, and with a level of significance of 1% (p=0.01), it was 2.58. The algorithm uses a p-value of 1.96 because, as is customary in research, p-values below 0.05 are assumed to be statistically significant. In this equation, p stood for the predicted population proportion according to earlier studies [7], and d for the researchers' documented absolute inaccuracy or precision. To summarise, depending on the type of illness, a sample was taken from either eye via having the patient look upward while lowering their eyelid. To delicately remove discharge from the eye, a sterile cotton swab was dampened with sterile normal saline before usage. Applying gentle pressure, the lower conjunctive sac was massaged from side to side and back again with the swab [8].

Isolation of S. aureus

The bacterial cultures were grown on various selective and differential media. These media were blood agar, MacConkey agar, and mannitol salt agar. The last medium is specific for the isolation of S. aureus bacteria. Incubation of the cultures was done at 37 °C for 24 hours. Identification of the bacteria was done via assessing the colony features of the bacteria.

Molecular work

A commercial kit (PrestoTM Mini gDNA Bacterial Kit, Geneaid, Thailand) was used to extract deoxyribonucleic acid (DNA) for use in polymerase chain reaction (PCR) experiments. We followed the manufacturer's instructions to extract DNA of the S. aureus isolates. The electrophoresis tank was prepared for electrophoresis on agarose gel via adding 1x Tris-borate-EDT (TBE) buffer. The agarose tray was then submerged in the tank. The buffer was made to sit a few millilitres above the surface of the agarose. The tank was filled and sealed after 5µl of specimen and 2 µl dye fluorescence were added to each well. A gel run under electrophoresis gradient of 70 volts/cm was used for the experiment. The agarose was removed off the tank and shown applying gel paper.

Primers were optimised via mixing 2.5 µl of master mix along with 5–6 µl DNA molecules and 1 µl of forward along with revers primers. Primers from different gene grades were selected, and the PCR annealing temperatures have been set at 55 °C, 58 °C, and 52 °C, respectively, for the SeA, Seb, Sec and Sed genes. Following the manufacturer's recommendations, a mixture of 12.5 ml master mix, 5–6 ml DNA, 1 ml reverse and forward primers, and 20 ml of nuclease-free deionised water was used to detect the selected genes. In order to identify the target genes, the PCR cycle program parameters were recorded (Table 1) [9].

Table 1
Primer sequences and amplification characteristics of enterotoxin genes detected via PCR

Gene	Primer name	Primer sequence (5'–3')	Gene location	Amplicon size (bp)
sed	F	CGAATAATAGGAGAAAA TAAAAG	492–514	278
	R	ATTGGTATTTTTTTCGTTC	750–769	
seb	F	GTATGGTGGTGTAACTGAGC	666–685	164
	R	CAAATAGTGACGAGTTAGG	810–829	
sea	F	GGTTATCAATGTGGGTGG	349–368	102
	R	CGGCACTTTTTTCTCTTCGG	431–450	
sec	F	AGATGAAGTAGTTGATGTGTATGG	432–455	451
	R	CACACTTTTAGAATCAACCG	863–882	

Notes: * F – Forward; R – Reverse.

Immunological work

The ELISA kits' methods are the Sandwich-ELISA. There are IL-6, IL-8 specific antibodies pre-coated on the microplate wells. According to the manufacturer's instructions.

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Statistical analysis

The data was analyzed applying the Statistical Package for the Social Sciences (SPSS), version 20. Chi-square test was employed to test the differences among categorical variables. A probability level of P<0.001 was considered statistically significant [10].

■ RESULT

Prevalence of bacteria among various eye infectious illnesses

Bacteria were recovered from 122 of the 213 ocular specimens that were processed for culture (57.27%). In this investigation, no mixed bacterial isolate per patient was discovered. Of the isolated bacteria, 94 (77.04%) belonged to the Gram-positive groups, while the remaining 28 (22.95%) belonged to the Gram-negative groups. S. aureus accounted for 54 isolates (27.9%) from the previous groups, with CoNS and S. pneumoniae with 30 (19.7%) and 8 (8.8%) isolates, respectively. P. aeruginosa was the most common isolate from the latter groups, accounting for 9 (6.8%), followed via K. pneumoniae with 8 (6.1%). The age of the patients affects the range of the bacterial isolate. Table 2 shows that the majority of the bacterial isolates were obtained from patients aged one month to two years.

Table 2
Distribution of Gram-positive bacterial isolates among different age groups of patients with ocular infectious illnesses

Bacterial isolate	0–2 years	3–16 years	17–45 years	>45 years	Total
S. aureus	20	10	12	12	54
Coagulase-negative staphylococci (CoNS)	10	6	7	2	25
Streptococcus pneumoniae	5	5	1	0	8
Streptococcus pyogenes	2	0	1	1	4
Streptococcus agalactiae	1	1	0	1	3

Prevalence S. aureus among different types of eye infectious illnesses

The prevalence of S. aureus among different eye infectious illnesses was displayed in Table 3. Conjunctivitis 23 (43%) had the largest percentage of S. aureus infectious illnesses in the current investigation, these bacteria can be regarded as one of the main agents of community-acquired S. aureus infectious illness in eye disease. Blepharoconjunctivitis was next, with S. aureus infectious illnesses occurring there most frequently 15 (27%), then blepharitis 8 (15%), dacryocystitis, 5 (9%), While the lower incidence was 3 (6%) in external eye infectious illnesses.

Table 3
Clinical distribution of S. aureus isolates among different types of ocular infectious illnesses

Type of ocular infectious illness	Number of isolates	Percentage (%)
Conjunctivitis	23	36
Blepharoconjunctivitis	15	24
Blepharitis	8	16
Dacryocystitis	5	13
Other external ocular infectious illnesses	3	5
Total	54	

Note: * Highly significant difference (P<0.01).

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Conventional PCR screening for virulence gens of S. aureus

All 54 S. aureus isolates were screened for the presence of staphylococcal enterotoxin genes A–D (sea, seb, sec, and sed). The outcomes, presented in Figure 1 and Figure 2, demonstrated variable distribution patterns of these virulence determinants. The sea gene showed the highest prevalence (94.5%), followed via seb (39.68%), sec (34.92%), and sed (28.57%).

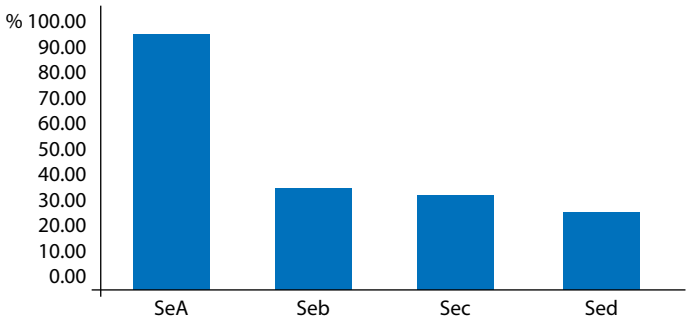

Fig. 1. Prevalence of virulence-related genes of S. aureus

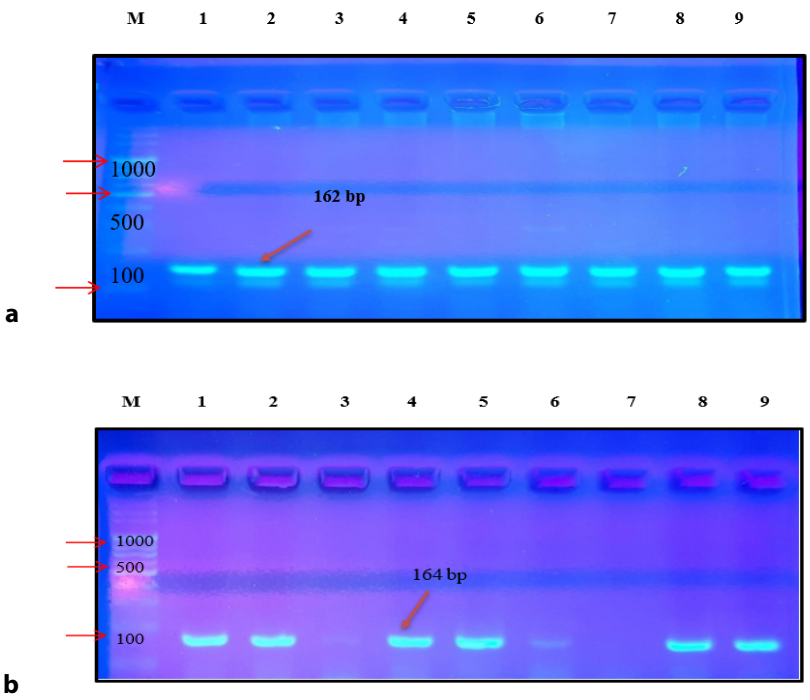

Fig. 2: Agarose gel electrophoresis for amplification of (a) 162 bp bands of sea gene (1–9 lanes positive); b) 164 bp of seb gene (1, 2, 4, 5, 8 and 9 lanes positive) of S. aureus. M: Marker ladder (50-1000 bp)

Levels of interleukin IL-6, IL-8 in patients with S. aureus infectious illness

Serum IL-6, and serum IL-8 were significantly elevated compared to controls in infected with S. aureus group, whereas Serum IL-6 (83.89±1.68) and Serum IL-8 (206.81±16.61), were significantly higher in infected with S. aureus infectious illness group as compared to Control (67.82±4.28 and 78.45±6.36); however, there was a significant difference (P<0.05**)** in serum concentration of IL-8 and IL-6 (Table 4).

Table 4
Comparison of serum IL-8 and IL-6 concentrations among patients with ocular infectious illness and healthy controls

Work groups	IL-6 (pg/mL)	IL-8 (pg/mL)
Control	67.82±4.28	78.45±6.36
Patients	83.89±1.68	206.81±16.61
t-test	3.98	7.21
P value	<0.0001	<0.0001

■ DISCUSSION

This outcome is similar to one from an earlier work carried out in Iraq [11]. Nevertheless, the outcome is less than the prevalence-which has been recorded from other sources and ranges from (74% to 88%) [12]. This resulted of work is agree to a work with [13]. S. aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus mirabilis, Citrobacter freundii, and Streptococcus spp. were identified as causative agents of eye infectious illnesses. Additionally, a work via [14] reported that (9%) of cases of conjunctivitis were caused via S. pneumoniae. The high prevalence of S. aureus infectious illnesses can be attributed to their normal presence on the skin as part of the normal flora. However, they can become pathogenic in some situations, such as immunocompromised individuals with long-term illnesses, and can result in infectious illnesses of the eyelids, cornea, and conjunctiva. These bacteria can also be transmitted via contaminated hands, and have virulence factors such as enzymes and toxins (e.g., protease and lipase). S. epidermidis is also part of the normal skin flora, but can cause infectious illnesses in hosts with weakened immune systems [15]. The presence of various bacterial species causing external ocular infectious illnesses indicates variations in environmental conditions, age, personal practices of hygiene, and the location of infectious illness. The low occurrence of Gram-negative enteric bacteria in this work may be attributed to effective personal hygiene since the primary transmission mode for enteric pathogenic organism is through fecal-oral contamination of the eye. Furthermore, previous studies have shown that wearing contact lenses is the main cause of Gram-negative bacterial ocular infectious illnesses [16]. Some of the patients in the current work had a history of contact lens wearing 9 (16%).

Findings from this investigation indicated that S. aureus obtained from ocular infections may serve as a source of opportunistic pathogens associated with ophthalmic disorders. Therapeutic administration of antibiotics for eye-related or systemic infections may disrupt the normal microbial balance, thereby promoting the proliferation of staphylococcal species within the ocular environment. Moreover, many S. aureus isolates exhibit substantial antimicrobial resistance, and this characteristic can contribute to the emergence or persistence of ophthalmic complications following antibiotic exposure. The increased occurrence of S. aureus in eye infections may therefore be associated with

more pronounced disease severity and poorer clinical outcomes. The current percentages of isolated S. aureus are consistent with those reported via [17], who discovered that 36 (33.8%) had conjunctivitis, while 19 (26.8%) had blepharitis and 12 (16.9%) had external eye infectious illnesses. Additionally, in line with the results of [18], 110 patients presenting to a hospital with a range of eye ailments showed a prevalence of S. aureus in the blepharoconjunctiviti of (21%) and conjunctivitis of (11%). Because of the variety of the normal flora and the healthy carriage of S. aureus in specific patient groups, the case for S. aureus in the pathogenesis of eye illness is difficult to make. However, doctors should take into account the possibility that this bacterium contributes to eye mucosal symptoms given the high frequencies of S. aureus recovery in patients with symptoms like pain, burning, erythema, and swelling. the frequency of S. aureus because the infectious illness of the exterior eye structures may have originated from a transient contamination of the patient's hand. Frequent hand and face washing with antimicrobial or nonantimicrobial soap is one of the sanitary and hygienic practices that can lower the number of bacteria that colonize the face, which in turn reduces the number of transitory organisms [19].

It has also been well documented that S. aureus is capable of producing a range of staphylococcal enterotoxins, abbreviated as SE, which have the potential to cause food poisoning-like symptoms after consumption. Apart from the toxicity, these enterotoxins have also been identified as significant virulence factors and may be responsible for the pathogenesis of toxic shock syndrome-like conditions. A previous local study identified the detection rate for the sea and seb genes at 86.78% and 52.2%, respectively [20, 21]. Consistent with these observations, the sea gene was found to be the most frequently detected enterotoxin gene compared with seb, sec, and sed, as also demonstrated in the present study. The toxic shock syndrome toxin (TSST), encoded via the tst gene [22], was identified in 35.13% of the examined S. aureus isolates. Regarding the sec gene, delta-hemolysin is a peptide consisting of 26 amino acids encoded via this locus, although the precise mechanism of delta-toxin secretion remains unclear [23]. The markedly high occurrence of the sea gene observed in this work (100%) is in agreement with previous findings that also reported similar detection rates [24, 25]. Variability in the distribution of S. aureus virulence genes may occur across different geographical regions, between cities within the same country, and even among hospitals or clinical departments within a single healthcare facility. Such heterogeneity has been attributed to differences in bacterial carriage patterns and environmental contamination in hospital settings, both of which may facilitate the development and transmission of infectious diseases.

A significant immune response, including the production of pro- inflammatory cytokines like interleukin-6 (IL-6) and interleukin-8 (IL-8), is induced via infectious illnesses with S. aureus. The results of this work demonstrate that levels of IL-8 and IL-6 are higher in S. aureus infected patients when contrasted with healthy controls. According to multiple studies, those infected with S. aureus show far greater levels of IL-6 in their bloodstream compared to people infected with other bacterial infectious illness [26]. Ngo et al. [27] found that keratinocytes secreted significantly more IL-8 and IL-6 than normal, which was a marker and indication of the severity of the infectious illness. An increased generation of pro- inflammatory cytokines was observed in animal models that contained MRSA, suggesting a robust immune response that may be linked to the virulence of S. aureus strains [28]. Extensive research has been conducted on the involvement of IL-6 to the disease processes of the eye. The absence of IL-6 has been linked to the worsening in

inflammation of the skin in a live model of mice [29]. Additionally, low levels of IL-6 in the eye have been connected to the worsening of atopic dermatitis, which is a skin disorder that is linked with a tendency for S. aureus colonization and infectious illness. Conversely, individuals with psoriasis have a lower likelihood of contracting S. aureus infectious illnesses. This skin condition is linked to increased levels of IL-6, which could serve as a possible focus for psoriasis treatment [30]. IL-6 have a vital function in connecting the innate and acquired immune responses in the skin's immunological function [31]. IL-8 have a function in attracting immune cells to start and continue the cascade of inflammatory responses. Several types of immune cells, including keratinocytes, endothelial cells, dermal macrophages, mast cells, T lymphocytes, and neutrophils, have IL-8 binding sites. The chemotactic capabilities have been ascribed to particular processes, including the migration of cells and wound healing [32]. Multiple investigations have demonstrated that an infectious illness of keratin cells with S. aureus trigger the production of IL-8 [33]. IL-8 has been shown to have a vital function in attracting neutrophils, which are necessary for eliminating germs, in a mouse model of S. aureus colonization/infectious illness [34]. The outcomes may have implications for comprehending the interaction among the host and bacterium, which could contribute to the establishment of S. aureus colonization. Previous research has shown that individuals who carry the bacteria S. aureus for a long time have lower levels of antimicrobial peptides, particularly human β defensin-3**(**hBD-3), compared to those who do not carry the bacteria. This difference in expression is linked with genetic variations in the DEFB1 gene [29]. Furthermore, the immune response mediated via Th1/Th17 cells in peripheral blood was less significant in individuals who carry S. aureus persistently compared to those who do not carry it, when stimulated with live S. aureus in a laboratory setting [35]. Septicemia is a condition characterized via the presence of bacteria in the bloodstream. The cytokine response, specifically IL-6, has been proposed as both a primary indication and a biomarker of intensity [26]. The response of cytokines in S. aureus bloodstream infectious illness similarly includes an increase in IL-6 initially in the infectious illness, even though the levels are lower compared to those observed in Gram-positive bacteria sepsis [37]. A previous work indicated that the levels of IL-6 decrease more quickly in those who survive sepsis compared to those who do not survive, within a 96-hour timeframe, thereby confirming its predictive significance [27]. Nevertheless, in the investigation via [38] on Septicemia caused via S. aureus, where cytokines were evaluated for an extended duration, the levels of IL-6 fell to comparable levels in both complex and uncomplicated cases of S. aureus infectious illness in the bloodstream via the seventh day after laboratory diagnosis. However, a notably increased IL-6 level at the time of diagnosing S. aureus bloodstream infectious illness seems to be linked with a worse outcome.

■ CONCLUSION

An increase in the levels of interleukin-6 and interleukin-8 was observed, but the significant and highly elevated increase in interleukin-8 is an indication of an immune response, as it haS an effective function in attracting cells to the site of infectious illness due to its binding regions for most immune cells.

REFERENCES

1. Mitiku A, Aklilu A, Biresaw G, Gize A. Prevalence and associated factors of methicillin-resistant Staphylococcus aureus among urinary tract infection-suspected patients attending Arba Minch General Hospital, Southern Ethiopia. *Infection and Drug Resistance.* 2021;14:2133–42.
2. Singh RK, Lokeshwar BL. The IL-8–regulated chemokine receptor CXCR7 stimulates EGFR signaling to promote prostate cancer growth. *Cancer Research.* 2011;71(9):3268–77.
3. Nishimoto N, Kishimoto T. Interleukin-6: from bench to bedside. *Nature Clinical Practice Rheumatology.* 2006;2(11):619–26.
4. Nurjadi D, Herrmann E, Hinderberger I, Zanger P. Impaired beta-defensin expression in human skin links DEFB1 promoter polymorphisms with persistent Staphylococcus aureus nasal carriage. *Journal of Infectious Diseases.* 2013;207(4):666–74.
5. Nurjadi D, Kain M, Marcinek P, et al. Ratio of T-helper type 1 to Th17 cytokines in whole blood is associated with human beta-defensin-3 expression in skin and persistent Staphylococcus aureus nasal carriage. *Journal of Infectious Diseases.* 2016;214(11):1744–51.
6. Al-Zoubi MS, Al-Tayyar IA, Hussein E, et al. Antimicrobial susceptibility patterns of Staphylococcus aureus isolated from clinical specimens in northern Jordan. *Iranian Journal of Microbiology.* 2015;7(5):265–72.
7. Sharma S. Antibiotic resistance in ocular bacterial pathogens. *Indian Journal of Medical Microbiology.* 2011;29(3):218–22.
8. Abd Zaid AM, Kandala NJ. Identification of Staphylococcus aureus using touchdown PCR and phenotypic methods from patients and hospital environments in different Iraqi cities. *Iraqi Journal of Agricultural Sciences.* 2021;52(6):1356–64.
9. Bae T, Banger AK, Wallace A, et al. Staphylococcus aureus virulence genes identified by bursa aurealis mutagenesis and nematode killing. Proceedings of the *National Academy of Sciences.* 2004;101(33):12312–7.
10. Okab HF, Salih MB, Jarulla BA. Evaluation of CXCL 10 and IL-10 in COVID-19 pneumonia. *Pneumologia.* 2024;71(4):175–80.
11. Salem AH, Ahmed EF, Darwish AMA, Gad GFM. Distribution of virulence genes among Staphylococcus aureus isolates causing wound infections and their correlation with antibiotic resistance. *BMC Infectious Diseases.* 2022;22(1):652.
12. Saleem AJ, Nasser NE, Ali MR. Potential bacterial pathogens of external ocular infections and their antibiotic susceptibility patterns. *African Journal of Microbiology Research.* 2015;9(14):1012–9.
13. Tesfaye T, Beyene G, Gelaw Y, et al. Bacterial profile and antimicrobial susceptibility patterns of external ocular infections at Jimma University Specialized Hospital, Southwest Ethiopia. *American Journal of Infection, Global and Microbiology*. 2013;1(1):13–20.
14. Aklilu A, Bitew A, Dessie W, et al. Prevalence and antimicrobial susceptibility pattern of bacterial pathogens from ocular infections at St. Paul's Hospital Millennium Medical College, Ethiopia. *Journal of Bacteriology and Mycology*. 2018;5(8):1085.
15. Al-Shably MK. Isolation and identification of bacteria causing eye infections in Diwaniya city. *Journal of College of Education.* 2011.
16. Hameed FA. Bacteriological study of eye infections in Baghdad city. *Indian Journal of Forensic Medicine & Toxicology.* 2020;14(3):183–7.
17. Epling J. Bacterial conjunctivitis. *BMJ Clinical Evidence.* 2012;2012:201.
18. Piette A, Verschraegen G. Role of coagulase-negative staphylococci in human disease. *Veterinary Microbiology.* 2009;134(1–2):45–54.
19. Hemavathi SP. Profile of microbial isolates in ophthalmic infections and antibiotic susceptibility patterns in an eye care hospital, Bangalore. *Journal of Clinical and Diagnostic Research.* 2014;8(1):23–5.
20. Zegans ME, Becker HI, Budzik J, O'Toole G. Role of bacterial biofilms in ocular infections. *DNA and Cell Biology.* 2002;21(5–6):415–20.
21. Li X, Fang F, Zhao J, et al. Molecular characteristics and virulence gene profiles of Staphylococcus aureus causing bloodstream infections. *Brazilian Journal of Infectious Diseases.* 2018;22(6):487–94.
22. Azmi K, Qrei W, Abdeen Z. Screening of adhesion genes and biofilm production in methicillin-resistant Staphylococcus aureus isolates. *BMC Genomics.* 2019;20(1):578.
23. Yu F, Li T, Huang X, et al. Virulence gene profiling and molecular characterisation of hospital-acquired Staphylococcus aureus isolates associated with bloodstream infections. *Diagnostic Microbiology and Infectious Disease.* 2012;74(4):363–8.
24. Mirzaee M, Najar-Peerayeh S, Ghasemian AM. Detection of icaABCD genes and biofilm formation among clinical MRSA isolates. *Iranian Journal of Pathology.* 2014;9(4):257–62.
25. Degaim ZD, Shani WS, Hamim SS. Virulence factors of methicillin-resistant Staphylococcus aureus isolated from burn patients. *International Journal of Current Microbiology and Applied Sciences.* 2015;4(7):898–906.
26. McNicholas S, Talento AF, O'Gorman J, et al. Cytokine responses to Staphylococcus aureus bloodstream infection differ between patient cohorts. *BMC Infectious Diseases.* 2014;14(1):580.
27. Ngo QV, Faass L, Sähr A, et al. Inflammatory response against Staphylococcus aureus via intracellular sensing of nucleic acids in keratinocytes. *Frontiers in Immunology.* 2022;13:828626.
28. Haisma EM, Rietveld M, Breij A, et al. Inflammatory and antimicrobial responses to MRSA in an in-vitro wound infection model. *PLoS ONE.* 2013;8(12):e82800.
29. Lee BY, Singh A, David MZ, et al. Economic burden of community-associated MRSA. *Clinical Microbiology and Infection.* 2013;19(6):E149–56.
30. Croxford AL, Karbach S, Kurschus FC, et al. IL-6 regulates neutrophil microabscess formation in IL-17A-driven psoriasiform lesions. *Journal of Investigative Dermatology.* 2014;134(3):728–35.
31. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. *Annual Review of Immunology.* 2009;27:485–517.
32. Jiang WG, Sanders AJ, Ruge F, Harding KG. Influence of IL-8 and its receptors on keratinocyte migration. *Experimental and Therapeutic Medicine.* 2012;3(2):231–6.
33. Takada K, Komine-Aizawa S, Hirohata N, et al. Poly I:C induces collective migration of keratinocytes via IL-8. *BMC Immunology.* 2017;18(1):19.
34. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. Pro- and anti-inflammatory properties of interleukin-6. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research.* 2011;1813(5):878–888.
35. Olaru F, Jensen LE. Staphylococcus aureus stimulates neutrophil-targeting chemokine expression in keratinocytes. *Journal of Investigative Dermatology*. 2010;130(7):1866–76.
36. Feezor RJ, Oberholzer C, Baker HV, et al. Molecular characterization of the acute inflammatory response to gram-positive versus gram-negative bacterial infections. *Infection and Immunity.* 2003;71(10):5803–13.
37. Yao L, Lowy FD, Berman JW. Interleukin-8 gene expression in Staphylococcus aureus-infected endothelial cells. *Infection and Immunity.* 1996;64(8):3407–9.
38. Jekarl DW, Lee SY, Lee J, et al. Procalcitonin as a diagnostic marker and IL-6 as a prognostic marker in sepsis. *Diagnostic Microbiology and Infectious Disease.* 2013;75(4):342–7. Available from: https://doi.org/10.1016/j.diagmicrobio.2012.12.008

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Prime Boost HIV Vaccination with Recombinant Influenza Virus Vectors Stimulates Specific and Mucosal CD8$^+$ T Cell Immune Response in BALB/c Mice

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Abstract

Introduction. HIV/AIDS continues to be a significant medical problem worldwide. An effective and safe vaccine remains a high priority. Most HIV vaccine candidates to date have failed to elicit effective immune responses that are necessary to control HIV infection.
Purpose. to design new mucosal CD8+ T cells based HIV vaccine using recombinant influenza viruses to deliver HIV-1 $Gag_{197-205}$ and Tat_{17-25} epitopes in order induce specific mucosal HIV-1 CD8+ T cells to control HIV-1 replication and dissemination.
Materials and methods. This study project used influenza viruses as mucosal live vaccine vector to stimulate effective CD8+ T cell immunity. Recombinant influenza A virus, H3N2 (HK-X31) and H1N1 (A/PR8/8/34) expressing defined mouse HIV-1 CD8+ T cell epitopes (H-2Kd $Gag_{197-205}$ and H-2Kd Tat_{17-25}) in the neuraminidase (NA) stalk were generated using reverse genetics and administered as a prime boost vaccine within various mucosal routes of vaccination, intranasal-intranasal, intravaginal-intravaginal, intranasal-intravaginal and intravaginal-intranasal vaccination in BALB/C mice. Following those prime-boost vaccinations, tetramer and intracellular cytokine staining assays used for detection of specific CD8+ T cell immune response in harvested organs, spleen, bronchoalveolar lavage (BAL), mediastinal and inguinal lymph nodes
Results. An induction of CD8$^+$ T cells targeted H-2Kd$Gag_{197-205}$, compared to no CD8$^+$ T cell responses specific for H-2Kd Tat_{17-25} in recombined influenza-HIV vaccinated BALB/c mice. Also, comparable HIV and endogenous influenza specific CD8$^+$ T cell responses following intranasally-intranasally prime boost vaccination in harvested lymphoid tissues, spleen, bronchoalveolar lavage and mediastinal lymph nodes compared to inguinal lymph nodes which included a high proportion of specific CD8$^+$ T cell immune response following intravaginal-intravaginal prime boost infection.
Conclusion. Intranasal prime boost vaccination as one of the mucosal routes of vaccination using recombinant influenza viruses as mucosal viral vectors of HIV vaccine in BALB/c mice has an important role in stimulating both specific and mucosal CD8$^+$ T cells within a high level and these cells would be important for migration of mucosal specific CD8$^+$ T cells given the mucosal acquisition of HIV infection and control of HIV-1 virus dissemination through mucosal compartments.
Keywords: prime boost HIV vaccination, recombinant vaccine vector, mucosal CD8+ T cell, immune response, BALB/c mice

■ INTRODUCTION

More than 91.4 million individuals have been infected in HIV infection, with 40.8 million infected persons are living with it. Moreover, more than 2.6 million new cases of infection are reported every year [1, 2]. Most cases of infection are located in the developing world, and nearly half of these individuals have died, especially in under developing countries [3]. The generation of a safe and effective HIV-1 vaccine remains the most likely approach to control HIV infection in human [4]. There have been many trials to develop new HIV vaccine candidates during the last 30 years, including DNA vaccines, recombinant vectored and subunit vaccines, mostly in non primate animals, such as macaques. However, four candidate vaccines have been tested in human with no significant efficacy for 3 of these, but one Thailand in 2009 (RV144: phase III) elicited a moderate level of protection against HIV (31%). As mucosal surfaces of human and animal body are considered the main portal of entry for most pathogenic viruses, therefore the main target to control mucosal transmission of these viral infections is the induction of mucosal immune response [5]. Moreover, it is a highly recommended of effective HIV vaccines to be able for induction of specific CD8+ T cells responses by targeting highly conserved regions of HIV-1 virus, which would be more effective in control virus replication.

Also, HIV-1 specific $CD8^+$ T lymphocytes play an effective role in preventing replication of virus through mucosal surfaces, such as genital mucosa, resulting in control of HIV infection. For example, seronegative, resistant Kenyan sex workers have a high level of mucosal cytotoxic $CD8^+$ T cells, compared to infected groups of sex workers [6]. Also, it is indicated that HIV-1 resistant individuals are similarly characterized by high levels of granzymes and perforin, leading to high cytolytic activity of cytotoxic $CD8^+$ T cells. Additionally, there is a negative correlation between HIV-1 RNA copy number and the level of specific HIV-1 $CD8^+$ T cells [7].

Moreover, there is highly significant proportion of HIV-1 Gag+ mucosal $CD8^+$ T cells detected in rectal mucosa of non-viremic group of HIV-1 patients compared to another groups of HIV-1 chronic patient groups. This confirmed that the broadly mucosal HIV-1 Gag $CD8^+$ T cells in mucosal tissues induced against conserved regions of HIV Gag epitope has a critical role to control HIV-1 viral load compared to another parts of body [8]. Also, as a response to high conserved regions of HIV-1 Gag epitopes, broadly Gag+ CD8+ T cells are controlling viremia in HIV-1 infected individuals compared to $CD8^+$ T cells induced in response to variable regions of HIV-1peptides [9]. According to all previous mentioned roles of HIV CD8+ T cell in control of HIV-1 replication and transmission, therefore our project's goal was to design new mucosal $CD8^+$ T cells based HIV vaccine using recombinant influenza viruses to deliver HIV-1 $Gag_{197\text{-}205}$ and $Tat_{17\text{-}25}$ epitopes in order induce specific mucosal HIV-1 $CD8^+$ T cells to control HIV-1 replication and dissemination through mucosal compartment preferred by HIV virus entry.

■ MATERIALS AND METHODS

Rescue of recombinant influenza-HIV $Gag_{197\text{-}205}$ and influenza-HIV $Tat_{17\text{-}25}$

Two strains of laboratory influenza viruses, and X31 (H3N2) and PR8 (H1N1) have been used in the reverse genetics' strategy. Cloning work included a design of specific forward and reverse primers necessary for generation of HIV epitopes, such as $Gag_{197\text{-}205}$ AMQMLKETI (5'GCC ATG CAA ATG TTA AAA GAG ACC ATC 3') and $Tat_{17\text{-}25}$ QPKTACTNC (5'CAG CCT AAA ACT GCT TGT ACC AAT TGC 3', and amplification of new PR8-NA and X31-NA gene

segments inserted with the generated HIV-1 epitopes ($Gag_{197-205}$ and Tat_{17-25}), ligated with pHW2000 plasmid and used as one segment of the eighth segments of new modified X31 or PR8 influenza viruses.

The cloning work involved amplification with PCR for cloning sequences of $Gag_{197-205}$ into NA segment using specific designed primers, included forward primer for short fragment, Bm-NA-1–F: 5'TAT TCG TCT CAG GGA GCA AAA GCA GGA GT-3'; Reverse primer for short fragment , X31-NA197(45)R: 5'-GAT GGT CTC TTT TAA CAT TTG CAT GGC GGA GTC GCA CTC ATA TTG CTT AAA ATG CAA TG-3'; forward primer for large fragment, X31-NA197(45)F: 5'-GCC ATG CAA ATG TTA AAA GAG ACC ATC CCC GCG AGC AAC CAA GTA ATG CCG TGT GAA-3'; and reverse primer for large fragment, Bm-NA-1413R: 5'ATA TCG TCT CGT ATT AGT AGA AAC AAG GAG TTT TTT-3'

Detection of recombinant influenza viruses in harvested allantoic fluids

RNAs were extracted using the RNeasy Mini Kit (Qiagen) as mentioned previously by [10]. After that, extracted RNAs were treated with RQ1 DNase (Promega) before converted to cDNA in order to avoid any genomic DNA of interference with IL-15 expression, and then, purified RNA of modified recombinant influenza viruses were reverse transcribed by adding the extracted RNA to an RNase- free PCR tube in a volume of 10 µl and to this was added 2 µl of 10× reverse transcriptase buffer (RT buffer), 2 µl dNTP mix, 2 µl of 10 µM oligo-dT primer (or BmNA-1 forward primer), and 1µl of Omniscript reverse transcriptase), and 3 µl RNA mixed well, before incubation for 60 min at 37 °C. An aliquot of the resultant cDNA (5 µl) was mixed with 10 µl of 1 × phusion master mix, 2 µl of each forward and reverse primer (BmNA-1 and BmNA-1413), and 1 µl of nuclease free water, and subject to the PCR2 cycling conditions (Table).

Table 1
Conditions for PCR reactions: A) Standard PCR conditions used in cloning work, B) Conditions for the recombinant PCR reactions to generate the modified NA gene segments

Step of reaction A) Standard PCR conditions	Temp.	Time	Cycle number
1	94 °C	4 min	1
2	94 °C	20 sec	30
3	57 °C	30 sec	
4	72 °C	2 min	
5	95 °C	1 min	1
6	57 °C	1 min	
7	72 °C	7 min	
8	4 °C	Hold	
Step of reaction B) Recombinant PCR conditions	**Temp.**	**Time**	**Cycle number**
1	94 °C	2 min	2
2	55 °C	30 sec	
3	72 °C	2 min	
4	94 °C	30 sec	33
5	59 °C	30 sec	
6	72 °C	2 min	
7	72 °C	7min	1
8	4 °C	Hold	

Vaccination and infection of BALB/C mice

Six-eight weeks old BALB/c mice were purchased from the Animal Resources Centre (ARC) and then housed under daily observation and monitoring program until adaptation before they inoculated with the recombinant influenza vaccines strains following environmental adaptation. The vaccination program followed various vaccine strategies to stimulate CD8 T cell responses. i) Intranasal administration of 30 µl (1×10^4 PFU) recombinant influenza vaccine (X31-NA-$Gag_{197-205}$) as a prime dose, followed 6 weeks later by intranasal immunization with 30 µl (50 PFU) PR8-NA-$Gag_{197-205}$. ii) Intravaginal administration of 30 µl (1×10^4 PFU) recombinant influenza vaccine (X31-NA-$Gag_{197-205}$) as a prime dose, followed 6 weeks later by intravaginal immunization with 30 µl (50 PFU) PR8-NA-$Gag_{197-205}$. iii) Intranasal administration of 30 µl (1×10^4 PFU) recombinant influenza vaccine (X31-NA-$Gag_{197-205}$) as a prime dose, followed 6 weeks later by intravaginal immunization with 30 µl (50 PFU) PR8-NA-$Gag_{197-205}$, and iv) Intravaginal administration of 30 µl (50 PFU) PR8-NA-$Gag_{197-205}$ as a prime dose, followed 6 weeks later by intranasal immunization with 30 µl (1×10^4 PFU) recombinant influenza vaccine (X31-NA-$Gag_{197-205}$).

Statistical analysis

The data of the present study was statistically analysis by using SPSS version 26, based in using independent sample t test and One way ANOVA at p. value [11].

■ RESULTS

Novel vaccine strain of recombinant influenza viruses expressing HIV-1 $Gag_{197-205}$

Many previous trials of vaccination procedures in mice and other laboratory animals have been applied using live recombinant vectors expressing specific genes inserted into these vectors by a novel strategy named reverse genetics reverse genetics. As one of the most recommended viruses used in this strategy, influenza A viruses were used to generate recombinant influenza viral vectors, in which specific functional exogenous and endogenous nucleotides of HIV $CD8^+$T cell epitopes inserted into the NA stalk of influenza viruses, such as PR8 (H1N1) and X31 (H3N2). In order to evaluate CD8+ T cell immune response induced in mice as a result of stimulated NP^+ and HIV specific epitopes, the amplified new generated recombinant influenza viruses expressed specific HIV-$Gag_{197-205}$ epitopes need to be titred for detection of the viral dose in order to use in primary and secondary influenza infection of BALB/C mice. Therefore, a sample of the harvested allantoic fluids was subjected to HA assay to confirm successful amplification of the rescued viruses. Also, all of these rescued viruses used in a plaque assay in order to detect the optimum dose of these viruses used in infection of mice.

In this study, influenza viruses were used as a live vector for delivering HIV-1 $Gag_{197-205}$ into mucosal areas through a prime boost infection in BALB/c mice in order to induce both $HIVGag_{197-205}$ and influenza $NP_{147-155}$ specific CD8+ T cell response. For evaluation of immuno-efficacy of the recombinant modified vaccine and its ability to induce specific immune response against HIV and influenza epitopes, various procedures of vaccination were performed in a mouse model in order to obtain which one of these procedures is the best procedure to enhance HIV specific and mucosal immune response. Accordingly, BALB/c mice were infected intranasally (i.n) as a primary infection using the modified X31-NA-$Gag_{197-205}$ and X31-NA-Tat_{17-25} individually in order to test the ability of single dose of the recombinant influenza-HIV vaccine to stimulate specific CD8+ T cell immune

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responses and according to results of induced CD8+ T cell immune response against $HIVGag_{197-205}$ following primary intranasal infection, there was no detected $HIVGag_{197-205}$+ CD8+ T cells response in the group of BALB/c mice of intranasally infected with X31-NA-Tat_{17-25} compared to a clearly detected immune response induced for $HIVGag_{197-205}$ in the intranasal primary infected mice with X31-NA-$Gag_{197-205}$ (data no shown). Therefore, we continue our plan of next experiment with X31-NA-$Gag_{197-205}$ and PR8-NA-$Gag_{197-205}$ only. While, the next step was the prime boost infection of BALB/c mice using the modified recombinant influenza-HIV vaccines within different routes of infection, intranasally or intravaginally as previously mentioned in the method section.

To characterise the quality of cellular immune response for both influenza $NP_{147-155}$ and $HIVGag_{197-205}$ specific $CD8^+$ T cells induced by different mucosal routes of vaccination including primary and secondary infection of BALB/C mice, the intracellular cytokine (ICS) was used.

■ DISCUSSION

As a result of failure of most traditional viral vaccines, such as whole killed or live attenuated virus vaccines to induce an appropriate immune response for protection against HIV-1 virus infection, new strategies are required to induce protective immune responses. One of these strategies is reverse genetics which involved the recombinant vectors delivered HIV-1 genes, peptides or epitopes to induce immune response necessary to control HIV-1 progression [12, 13]. Reverse genetics is a powerful strategy

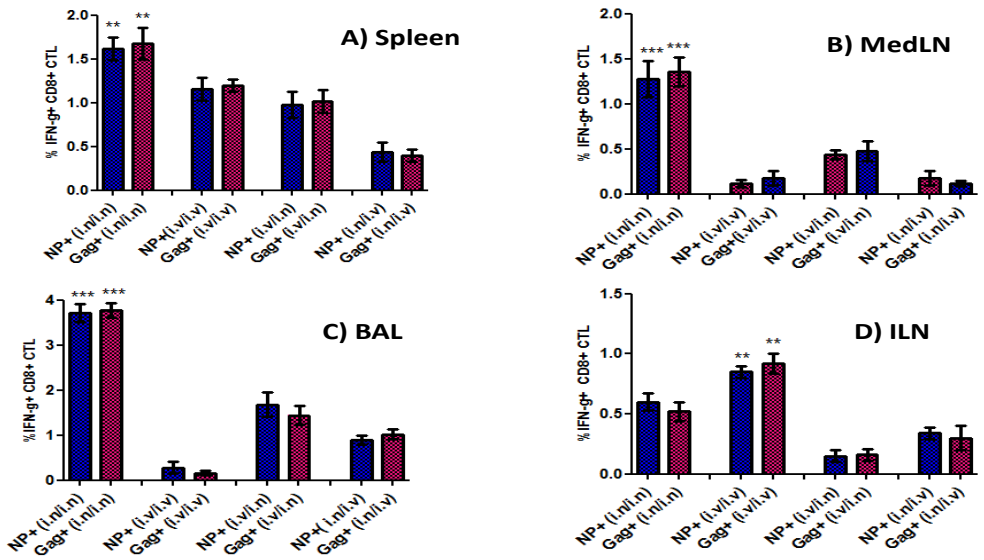

Fig. 1A. This figure showed that induced $CD8^+$ T cells specific for $Gag_{197-205}$ and $NP_{147-155}$ were equivalent as a proportion of specific $CD8^+$ T cells (Figure 1B) and the higher level of induced cells was detected following each last intranasal dose of infection, within a high significant difference following two intranasal doses of vaccination which was clearly detected in spleen (A), BAL (B) and mediastinal lymph nodes (C)

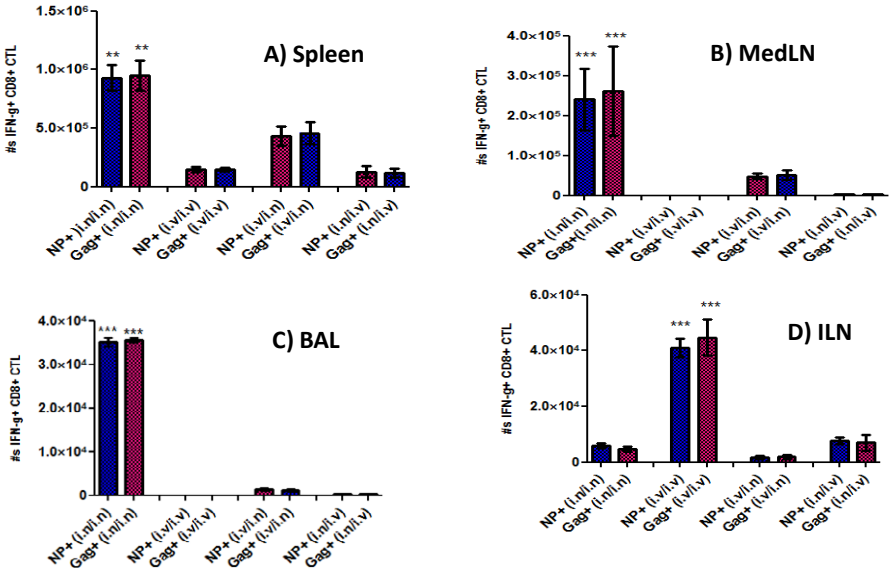

Fig. 1B. This figure showed that induced CD8⁺ T cells specific for $Gag_{197-205}$ and $NP_{147-155}$ were equivalent as number of specific CD8⁺ T cells and the higher level of induced cells was detected following each last intranasal dose of infection, within a high significant difference following two intranasal doses of vaccination which was clearly detected in spleen (A), BAL (B) and mediastinal lymph nodes (C)

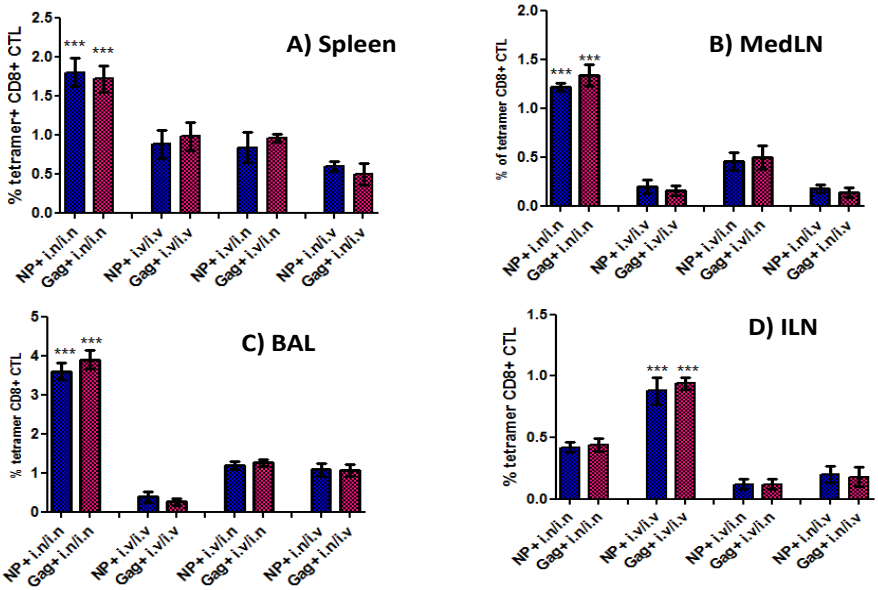

Fig. 2A. In this figure, there was an equivalent proportion of specific tetramer⁺ $HIVGag_{197-205}$ and influenza $NP_{147-155}$ CD8⁺ T cells induced in spleen and another pulmonary inflammatory regions or draining lymph nodes (as shown in Figure 2A and Figure 2B) which also showed detected levels of $HIVGag_{197-205}$ tetramer+CD8+ T cells induced as a response to different prime boost vaccination strategies, however the Intranasal prime boost administration of our vaccine gave the strongest responses in spleen (A), BAL (B) and MedLN (C)

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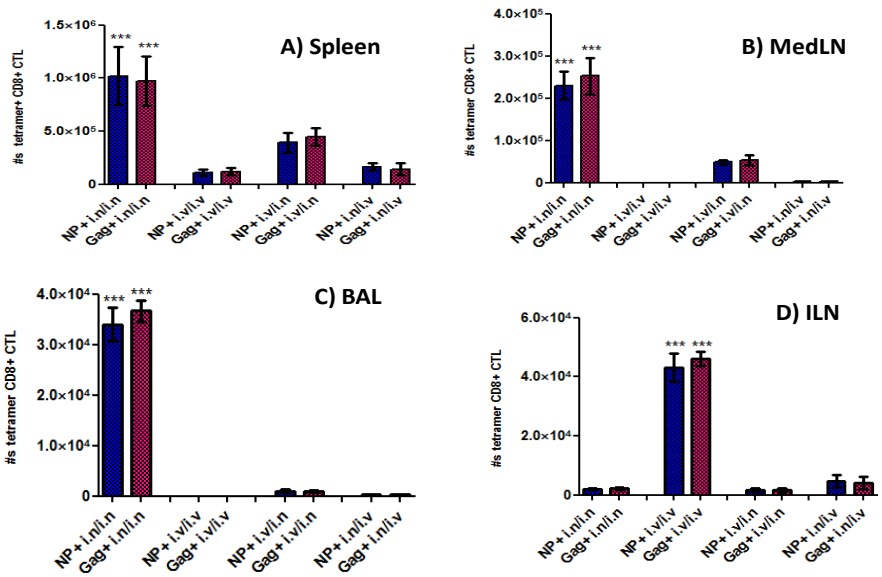

Fig. 2B. In this figure, there was an equivalent proportion of specific tetramer+ HIVGag$_{197-205}$ and influenza NP$_{147-155}$ CD8+ T cells induced in spleen and another pulmonary inflammatory regions or draining lymph nodes (as shown in Figure 2A and Figure 2B) which also showed detected levels of HIVGag$_{197-205}$ tetramer+CD8+ T cells induced as a response to different prime boost vaccination strategies, however the Intranasal prime boost administration of this vaccine gave the strongest responses in spleen (A), BAL (B) and MedLN (C)

used in a range of different applications, such as gene therapy and vaccines development. It involves manipulation of the specific genes, particular those of viral or bacterial agents, in order to create novel modified features. Also, reverse genetics has been applied to generate modified negative sense RNA viruses from cloned cDNA. Influenza viruses were similarly a meanable to this process to generate modified influenza viruses. This work has been extended to the use of the segmented genome of influenza virus as a vector for expressing specific antigens from other viruses to generate novel vaccines [5, 13, 14].

In this project, two HIV-1 epitopes were used to be delivered by two kinds of influenza viruses, X31 and PR8. First HIV-1 epitope derived from a highly conserved regions of group specific antigen, Gag$_{197-205}$ as a structural protein necessary for assembly of newly generated virion during life cycle of HIV-1 in infected individuals [15], and the another one is a Tat17-25 as a regulatory protein with the ability to transmit virus from infected cell to non-infected cell [16, 17] which would be targeted. In this study, Influenza virus type A has been used as a live vector as it is considered one of the most attractive candidates of efficient viral vector used for expressing viral antigens, especially those targeting respiratory viral infections and other mucosal transmitted diseases [16, 18]. This is because the ability of influenza virus to infect through the mucosal regions, resulting in a broad and long-term immune responses [19–21]. It is also relatively easy to manipulate gene segments of influenza virus by reverse genetics to generate novel recombinant viruses for use as vaccine vectors [22]. Most of the recombinant influenza viruses generated by reverse genetics have involved manipulation of HA and NA genes [23].

Previous studies of HIV vaccines in animal models were used different vectors to deliver specific HIV epitopes or proteins. For example, adenovirus, vaccinia virus, fowl pox viruses were used as vectors in various previous HIV-1 vaccine studies. All of these viral vectors used induced immune response in different levels; however, they were not protected against HIV. This is because they were not concentrated on stimulation of mucosal immune response, which is considered the direct way to control HIV transmission through mucosal surfaces. Therefore, in our study we focused on mucosal viruses to be used as a vector to deliver HIV epitopes. For this purpose, we chose influenza virus, this is because of the ability of influenza virus to induce a strong mucosal immune response particularly in the mucosal regions of the upper respiratory tract, which would be very sensitive to influenza virus infection. Moreover, there is a high link and strong connection between the mucosa of upper respiratory tract and of uro-genital tract mucosa, with mucosally-administered antigens able to illicit specific mucosal immune response in different mucosal surfaces [24]. Importantly, the two strains of laboratory influenza viruses were used previously to express HIV-1 $CD8^+$ T cell epitopes in mice for stimulation of specific CD8 T cell immune response against specific HIV Env epitope [25].

Following intranasal dose of infection, X31-NA-$Gag_{197-205}$ influenza infected BALB/c mice were showing a significant difference loss of weight started from the third day until the sixth day of infection which was suspected followed replication of influenza virus in respiratory tract mucosa as a result of protease enzyme in pulmonary mucosal area increasing the pathogenicity of influenza virus infection in compared to the intravaginal infected group of mice which not shown any detected loss of weigh as there is no enzyme effecting the pathogenicity of infected influenza virus, however, this is not means there was no induced immune response in the urogenital mucosa following influenza infection. Different parameters were highly recommended to use for detection of characterised and magnitude of specific CD8+ T cells following infection or vaccination. For example, IFN-γ+ production measured by intracellular cytokines and cytotoxicity assay, and the peptide tetramer staining assay are the mostly dominant methods used for measurement of immune response [26, 27].

In order to compare of different mucosal routes for prime boost infection in BALB/c mice, lymphocytes harvested from spleen, bronchoalveolar lavage, respiratory draining lymph nodes (mediastinal) and genital draining lymph nodes (inguinal) lymph nodes of various mucosal routes of immunized BALB/c mice were tested for production of cytokines, such as IFN-γ+, TNF-α and IL-2 specific for HIV-$Gag_{197-205}$ and influenza-$NP_{147-155}$ epitopes of harvested $CD8^+$ T cells stimulated five hours with each of Kd-HIV-Gag and Kd-flu-NP labelled peptides separately. Our results indicated that intranasal prime boost infection of BALB/c group of mice with the recombinant influenza viruses expressing HIV-$Gag_{197-205}$ induced a significant increase of producing IFN-g CD8+ T cells as proportion and number ($P<0.05$) in respiratory lymphoid regions, BAL and MedLN in addition to spleen in comparison to that of another groups of mice immunised by intravaginal–intravaginal, or by combination of intranasal-intravaginal prime boost vaccination. While in ILN, there is a significant increase in IFN-γ^+ produced specific HIV-Gag and NP^+ $CD8^+$ T cells after intravaginal-intravaginal prime boost infection compared to another routes of immunization. In our study, following influenza infection in mice, number of specific CD8+ T lymphocytes were detected by measuring the expressed IFN-γ+/CD8+ T cell induced in a response to specific influenza peptide stimulation, whereas function of T lymphocytes

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immune response is detected by measuring both TNF-α^+ and IL-2^+ producing CD8+ T cells. As subsets of functional T cells, TNF-α^+/$CD8^+$ T cell population is a subset of IFN-γ^+/$CD8^+$ T cell and subsequently IL-2^+ $CD8^+$ T cells is a smaller subset of TNF-α^+ $CD8^+$T cells population [28, 29]. Also, these data shown a high increase of IFN-γ^+/$CD8^+$ T cell against both Gag and NP+ peptides following prime boost intranasal infection in various lymphoid organs, spleen, BAL, MedLN compared to other procedures of prime boost infection. While, in ILN the intravaginal infection was the only procedure increases antigen specific CD8+ T cells producing IFN-γ^+ as the genital tract draining lymph nodes. As mentioned previously, both tetramer and ICS indicated to a detected $HIVGag_{197\text{-}205}{}^+$ and influenza $NP_{147\text{-}155}{}^+$ $CD8^+$ T cells responses in urogenital draining lymph nodes in addition to secondary lymphoid tissues, spleen is an evident that both influenza virus and $HIVGag_{197\text{-}205}$ antigens were recognised by specific CD8+ T cells and this is also supported by ability of influenza virus to replicate in the genital mucosa of progesterone treated mice following infection with a higher dose of influenza virus induced detected cytotoxic T cells in genital mucosa compared to lower dose of influenza virus led to low or not detected titer of replicated influenza virus with detected CTL response [30, 31].

■ CONCLUSION

According to results of this study, it is concluded that the intranasal prime boost vaccination as one of the mucosal routes of vaccination using recombinant influenza viruses as mucosal viral vectors of HIV vaccine in BALB/c mice has an important role in stimulating both specific and mucosal CD8+ T cells against each of $Gag_{197\text{-}205}$ and influenza-$NP_{147\text{-}155}$ epitopes in compared to HIV-Tat, within a high level and these cells would be important for migration of mucosal specific CD8+ T cells given the mucosal acquisition of HIV infection and control of HIV-1 virus dissemination through mucosal compartments.

■ REFERENCES

1. Françoise Barré-Sinoussi, Jean-Claude Chermann, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science.* 1983;220(4599):868–71.
2. World Health Organization. Global Health Observatory: HIV/AIDS. Geneva: WHO; 2025. Available from: WHO Global HIV Observatory.
3. Duerr A, Wasserheit JN, Corey L. HIV vaccines: new frontiers in vaccine development. *Clin Infect Dis.* 2006 Aug 15;43(4):500–511.
4. Alex C. Boomgarden, Chitra Upadhyay. Progress and challenges in HIV-1 vaccine research: a comprehensive overview. *Vaccines (Basel).* 2025;13(2):148. doi: 10.3390/vaccines13020148
5. Shim I, Rogowski L, Venketaraman V. Progress and recent developments in HIV vaccine research. *Vaccines (Basel).* 2025;13(7):690.
6. Hu JK, Crampton JC, Cupo A, et al. Murine immunity and antibody responses to HIV-1 envelope immunogens at mucosal sites. *Front Immunol.* 2023;14:1175234.
7. Hersperger AR, Migueles SA, Betts MR, Connors M. Qualitative features of the HIV-specific CD8+ T-cell response associated with immunologic control. *Curr Opin HIV AIDS.* 2011;6(3):169–173.
8. Gordon SN, Cervasi B, Odorizzi P, et al. Disruption of intestinal CD4+ T-cell homeostasis is a key marker of systemic CD4+ T-cell activation in HIV-infected individuals. *J Immunol.* 2010;185(9):5169–79.
9. Kunwar P, Hawkins N, Dinges WL, et al. Superior control of HIV-1 replication by CD8+ T cells targeting conserved epitopes: implications for HIV vaccine design. *PLoS One.* 2013;8(5):e64405.
10. Okab HF, Salih MB, Jarulla BA. Evaluation of CXCL 10 and IL-10 in COVID-19 pneumonia. *Pneumologia.* 2024;71(4):175–180.
11. Watanabe T, Zhong G, Russell CA, et al. A comprehensive deep sequencing strategy for full-length genomes of influenza A viruses. *PLoS One.* 2011;6(5):e19075.
12. Douek DC, Kwong PD, Nabel GJ. The rational design of an AIDS vaccine. *Cell.* 2006;124(4):677–681.
13. Manafi MM, Farzani T, Espinoza N, et al. Understanding the performance of HIV-1 viral vector vaccines: adenovirus and poxvirus case studies. *Front Immunol.* 2025;16:1720342.
14. Govindan R, Stephenson KE. HIV vaccine development at a crossroads: new B and T cell approaches. *Vaccines (Basel).* 2024;12(9):1043.
15. Sundquist WI, Kräusslich HG. HIV-1 assembly, budding, and maturation. *Cold Spring Harb Perspect Med.* 2012;2(7):a006924.
16. Govindan R, Stephenson KE. HIV vaccine development at a crossroads: new B and T cell approaches. *Vaccines (Basel).* 2024;12(9):1043.
17. Gavioli R, Cellini S, Castaldello A, et al. The Tat protein broadens T cell responses directed to the HIV-1 antigens Gag and Env: implications for the design of new vaccination strategies against AIDS. *Vaccine.* 2008;26(5):727–737.

18. Barouch DH. Challenges in the development of an HIV-1 vaccine. *Nature.* 2008;455(7213):613–619.
19. Krammer F, Smith GJD, Fouchier RAM, et al. Influenza. *Nat Rev Dis Primers.* 2018;4:3.
20. Neumann G, Ozawa M, Kawaoka Y. Reverse genetics of influenza viruses. *Methods Mol Biol.* 2012;865:193–206.
21. Neumann G, Kawaoka Y. Influenza virus reverse genetics: generation of influenza viruses from cloned cDNAs. *Rev Med Virol.* 2002;12(1):13–30.
22. Ussell CJ, Hu M, Okda F. Influenza hemagglutinin protein stability, activation, and pandemic potential. *J Mol Biol.* 2018;430(21):3733–3752.
23. Dou D, Revol R, Östbye H, et al. Influenza A virus cell entry, replication, virion assembly and movement. *Front Immunol.* 2018;9:1581.
24. Czerkinsky C, Holmgren J. Mucosal delivery routes for optimal immunization: targeting immunity to the right tissues. *Curr Top Microbiol Immunol.* 2012;354:1–18.
25. Sexton A, De Rose R, Reece JC, et al. Evaluation of recombinant influenza virus vectors expressing HIV-1 antigens as mucosal vaccines. *Vaccine.* 2010;28(9):1939–1946.
26. Borrow P, Harty JT, Zhao J. The immune response to HIV: challenges and opportunities for vaccine development. *Nat Rev Immunol.* 2020;20:1–14.
27. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med.* 2012;366(14):1275–1286.
28. Koutsakos M, Illing PT, Xu H, et al. Immunological outcomes of influenza infection and vaccination: understanding the role of T cells. *Nat Rev Immunol.* 2019;19(6):355–370.
29. Swain SL, McKinstry KK, Strutt TM. Expanding roles for CD4 T cells in immunity to viruses. *Nat Rev Immunol.* 2012;12(2):136–148.
30. Clements DE, Belliot G, Lapp K, et al. Influenza virus-based vaccine vectors: applications for infectious disease vaccine development. *Vaccine.* 2014;32(46):6075–6081.
31. Pushko P, Tretyakova I, Hidajat R, et al. Influenza virus-like particles as vaccine candidates: recent advances and future perspectives. *Vaccines (Basel).* 2021;9(5):463.

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Comprehensive Molecular and Phenotypic Analysis of Biofilm Inhibition in Staphylococcus Aureus: Impact of Doxorubicin HCl, Sonidegib, Sciadopitysin, and Hinokiflavone on the sigB and ica Operon Regulation

Conflict of interest: nothing to declare.
Authors' contribution: Aqeel Kadhim Hasan – conceptualization, data curation, investigations, methods, project administration, resources, software, validation, visualization, writing – original draft and writing – review & editing; Wathiq Abbas Hatite Al-Daraghi – conceptualization, data curation, project administration, resources, software, supervision, validation, and writing – review & editing.
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Abstract

Introduction. Staphylococcus aureus is a major opportunistic pathogen capable of forming robust biofilms and developing resistance to conventional antibiotic therapies, which contributes significantly to persistent and recurrent infections. Targeting biofilm-associated regulatory pathways represents a promising strategy for controlling bacterial virulence.
Purpose. This study aimed to evaluate the anti-biofilm activity of Doxorubicin HCl (DOX), Sonidegib (SNG), Sciadopitysin (SCP), and Hinokiflavone (HNF) and to investigate their effects on the expression of the biofilm-associated genes sigB, icaA, and icaB in Staphylococcus aureus.
Materials and methods. Biofilm formation was assessed using the crystal violet microtiter plate assay following treatment with the selected compounds. Gene expression analysis of sigB, icaA, and icaB was performed using quantitative real-time PCR (qPCR) to evaluate the molecular effects of treatment on biofilm regulatory pathways.
Results. Phenotypic analysis revealed that DOX and SNG exhibited minimal or no inhibitory effects on biofilm formation ($p>0.05$). In contrast, SCP and HNF significantly reduced biofilm biomass at 512 µg/mL by approximately 93.4% ($p=0.0358$) and 88.8% ($p=0.0421$), respectively. qPCR analysis demonstrated significant downregulation of sigB and icaA expression, with fold changes of 0.659 and 0.642, corresponding to reductions of 34.1% and 35.8%, respectively. Conversely, icaB expression was significantly upregulated, showing a fold change of 2.218 (121.8% increase), suggesting a potential compensatory adaptive response by the bacteria.
Conclusion. Sciadopitysin and Hinokiflavone demonstrated significant anti-biofilm and anti-virulence activities against Staphylococcus aureus by reducing biofilm formation and modulating key biofilm-associated genes, highlighting their potential as promising candidates for novel anti-biofilm therapies.
Keywords: biofilm, hinokiflavona, icaA, Sciadopitysin, sigB, Staphylococcus aureus

■ INTRODUCTION

The studied bacteria, Staphylococcus aureus, are the most dangerous human pathogens, as they are able to cause a wide spectrum of diseases, ranging from benign skin infections to life-threatening conditions such as sepsis and endocarditis [1, 2]. The ability to form biofilms is an essential determinant of its pathogenicity and resistance to antibiotic therapy. Biofilms consist of single bacteria organized in specific structures that are protected from antimicrobial agents and host immunological responses by an extracellular polymeric substance (EPS) matrix [3]. Because of the rise in multidrug-resistant S. aureus strains with multigenic carriage (MRSA): Methicillin-Resistant S. aureus [4]. In S. aureus, the alternative global stress-response σ σB factor (SigB) regulates expression of many genes related to biofilm formation, metabolic adaptation, and virulence [5, 6]. The ica operon (icaADBC) is the locus primarily involved in biofilm formation and encodes for these enzymes that are essential to generate the polysaccharide intercellular adhesin (PIA). A key component of biofilm matrix [7]. In more detail, icaA codes for N-acetylgluco saminyltransferase, which polymerizes units of N-acetylglucosamine composing PIA, and icaB deacetylates PIA, modifying the negative charge and stability of the biofilm [8]. Such regulatory and structural genes would therefore provide a beneficial target to perturb the formation of biofilm, thereby diminishing bacterial virulence.

Natural products are recently shown as possible candidate agents to utilize as anti-biofilm. By way of example, studies have shown that Ginkgo biloba exocarp extracts can inhibit MRSA and S. aureus biofilms by interfering with their formation and modulating gene expression (including downregulation of icaA and sigB) [9]. In addition, hinokiflavone has been identified as a reducer or preventer of the S. aureus ClpP (caseinolytic protease) protein, which is important to bacterial homeostasis and pathogenicity [10], indicating that it can be used as a potential adjuvant for S. aureus infection control. The other phenolic compound, sciadopitysin, also showed the possibility to be an MRSA inhibitor targeting penicillin-binding protein 2A (PBP2a) [11]. Altogether these results support the examination of natural products as functional material to inhibit S. aureus biofilm-associated infections.

The present study was designed for a global perspective to assess the complex effects of four structurally distinct compounds (Doxorubicin HCl, DOX; Sonidegib, SNG; Sciadopitysin, SCP; and Hinokiflavone, HNF) on biofilm structure as well as the transcriptional dynamics at both sigB (the master regulatory gene controlling biofilm formation) and members of the structural ica operon (icaA and icaB). Our goal is to discover new anti-virulence agents that will help in the treatment of chronic infections with S. aureus by explaining the molecular mechanisms responsible for their actions.

■ MATERIALS AND METHODS

Supplements materials

All solutions employed in the current study and all reagents and culture media were purchased from commercial suppliers as indicated. Key materials included Mannitol Salt Agar (Oxoid, England), Brain Heart Infusion broth (Difco, U.S.A.), Luria Bertani broth (Oxoid, England), and other molecular biology reagents: Promega, USA; Geneaid, Thailand; and Alpha DNA, Columbia. The purification of DNA-Genome was performed by specific kit for bacteria (Presto™ Mini gDNA), RNA purification utilized the TriRNA Pure Kit (Geneaid Corporation).

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Specimen Collection and Bacterial Isolation

Between January and April 2024, 200 clinical specimens (anterior nares, wound swabs, burn swabs, mid-stream urine, ear swabs, sputum) were collected from patients in three Babylon hospitals (Al-Imam Al-Sadeq, Al-Hillah Teaching, Al-Qassim General). Swabs were transported in Stuart transport media [12]. After being inoculated onto Mannitol Salt Agar (MSA), the specimens were incubated for one day at a suitable bacterial temperature of growth temperature. Colonies of presumed S. aureus were purified on Brain Heart Infusion agar.

Identification and antimicrobial susceptibility testing

Bacterial isolates were identified based on morphological features and biochemical tests (Gram staining, catalase, oxidase, coagulase, acetoin production, hemolysin production) as described by [13–15]. Molecular confirmation was performed by amplifying the S. aureus species-specific Sa442 gene [16]. Antimicrobial susceptibility testing and species identification were further confirmed by VITEK 2 system, with card for Gram-positive test, including antibiotics from beta-lactams, aminoglycosides, fluoroquinolones, macrolides/lincosamides, glycopeptides, oxazolidinones, and tetracyclines.

Bacterial preservation

Isolates were preserved for short-term storage on nutrient agar slants at 4–8 °C and for long-term storage at minus 20 °C in tubes contain broth of brain heart infusion supplemented with glycerol about a 20% concentration, following [17].

Assay for biofilm formation

Microtiter plate was dependent on the biofilm formation assay, which was quantified as described by [18]. Briefly, 96-well polystyrene microplates were inoculated with bacterial suspensions (McFarland standard no. 0.5) in LB broth supplemented with 1% glucose and incubated at a suitable bacteria temperature for growth for one day. Then washed with PBS, followed by methanol fixation, next stained with 0.1% crystal violet, and finally re-solubilized with 33% glacial acetic acid. OD was detected at 600 nm by the reader. The cut-off value was calculated as the mean OD of control wells plus three standard deviations.

Quantitative Real-Time PCR (qPCR)

The level of Genomic expression for sigB, icaA, and icaB were quantified by qPCR. The housekeeping gene rpoB served as an internal control. Primar sequences are described in Table 1.

The results analysis statistically

The results are analyzed statistically as mean ± standard error (SE), and each experiment was conducted in triplicate. A p-value under 0.05 was deemed statistically significant, and statistical significance was assessed using Student's t-test or one-way ANOVA, if suitable. The 2^−ΔΔCt technique was used to calculate changes in gene expression fold [20].

Table 1
Primers used for PCR and gene expression analysis

Target Gene	Primer	Direction (5′ → 3′)	The Size of Amplicon (bp)	Reference
Sa442	SA1	AATCTTTGTCGGTACACGATATTCTTCAC	108	[16]
	SA2	GCGTAATGAGATTTCAGTAGATAATACAACA		
icaA	icaA-F	CAATACTATTTCGGGTGTCTTCACTCT	102	[19]
	icaA-R	CAAGAAACTGCAATATCTTCGGTAATCAT		
sigB	SigB-F	AGTGAGCGATGAACTAACCG	207	Current study
	SigB-R	TGGTCATCTTGTTGCCCCAT		
icaB	IcaB-F	TGAAACACATACCCACGATTTGC	207	Current study
	IcaB-R	TCGGAGTGACTGCTTTTTCCT		
rpoB	rpoB-F	CAGCTGACGAAGAAGATAGCTATGT	82	[19]
	rpoB-R	ACTTCATCATCCATGAAACGACCAT		

■ RESULTS

Prevalence and biofilm formation capacity of S. aureus

A total of 32/200 S. aureus isolates were identified from clinical specimens collected in Babylon Governorate hospitals, representing a prevalence of 16.0%. The isolates were predominantly recovered from wound specimens (13 isolates), followed by blood (9), urine (6), and respiratory samples (4) (p=0.0148). Identification was confirmed by standard biochemical tests and molecular amplification of the Sa442 gene (108 bp).

The capacity of biofilm formation assessed by microtiter plate assay. All 32 isolates (100%) were biofilm producers, with a significant dominance of strong producers (84.4%, 27/32), followed by weak (12.5%, 4/32) and intermediate producers (3.1%, 1/32) (p<0.0001).

Inhibitory effect of tested compounds on biofilm formation

The activity as anti-biofilm for Doxorubicin HCl (DOX), Sonidegib (SNG), Sciadopitysin (SCP), and Hinokiflavone (HNF) was evaluated at 64 and 512 µg/mL (Table 2).

DOX and SNG did not significantly inhibit biofilm formation; instead, they showed a non-significant trend toward increasing biofilm biomass (p>0.05). Conversely, SCP and HNF exhibited potent, concentration-dependent inhibitory effects. At 512 µg/mL, SCP

Table 2
Effects of DOX, SNG, SCP, and HNF on bacterial biofilm form

Compound	Con. (µg/mL)	OD of Biofilm (Mean±SE)	p-value (vs. Control)
Control	–	1.07±0.40	–
Doxorubicin HCl (DOX)	64	2.12±0.36	0.1144
	512	1.87±0.29	0.2567
Sonidegib (SNG)	64	1.47±0.45	0.7842
	512	1.47±0.46	0.7834
Sciadopitysin (SCP)	64	0.72±0.18	0.5896
	512	0.07±0.04	0.0358*
Hinokiflavone (HNF)	64	0.85±0.16	0.8050
	512	0.12±0.02	0.0421*

Note: p≤0.05 indicates statistical significance.

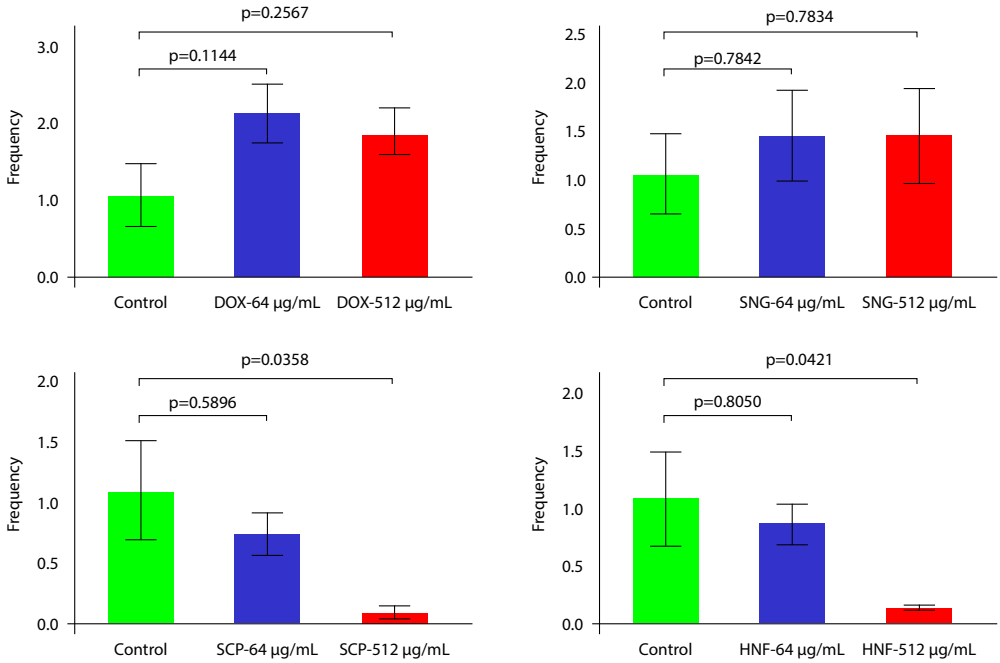

Fig. 1. Inhibitory effects of tested compounds on S. aureus biofilm formation

significantly reduced biofilm biomass to a mean OD of 0.07±0.04 (p=0.0358), and HNF reduced it to 0.12±0.02 compared to the control (1.07±0.40) with (p=0.0421). The obtained results are illustrated in (Fig. 1).

Transcriptional impact on biofilm-associated genes

To clarify the chemical processes behind SCP's and HNF as anti-biofilm properties, the expression of the master regulator sigB and the structural genes icaA and icaB was quantified by qPCR (Table 3).

Table 3
Gene expression analysis of sigB, icaA, and icaB following treatment

Gene	Ct (HKG)	Ct (Target)	ΔCt	ΔΔCt	Fold Change (2^-ΔΔCt)
sigB					
Control	14.93	19.66	4.73	0.00	1.000
Treatment	14.85	19.07	4.22	–0.51	0.659
icaA					
Control	14.93	24.20	9.27	0.00	1.000
Treatment	14.85	23.95	9.10	–0.17	0.642
icaB					
Control	14.93	24.20	9.27	0.00	1.000
Treatment	14.85	23.95	9.10	–0.33	2.218

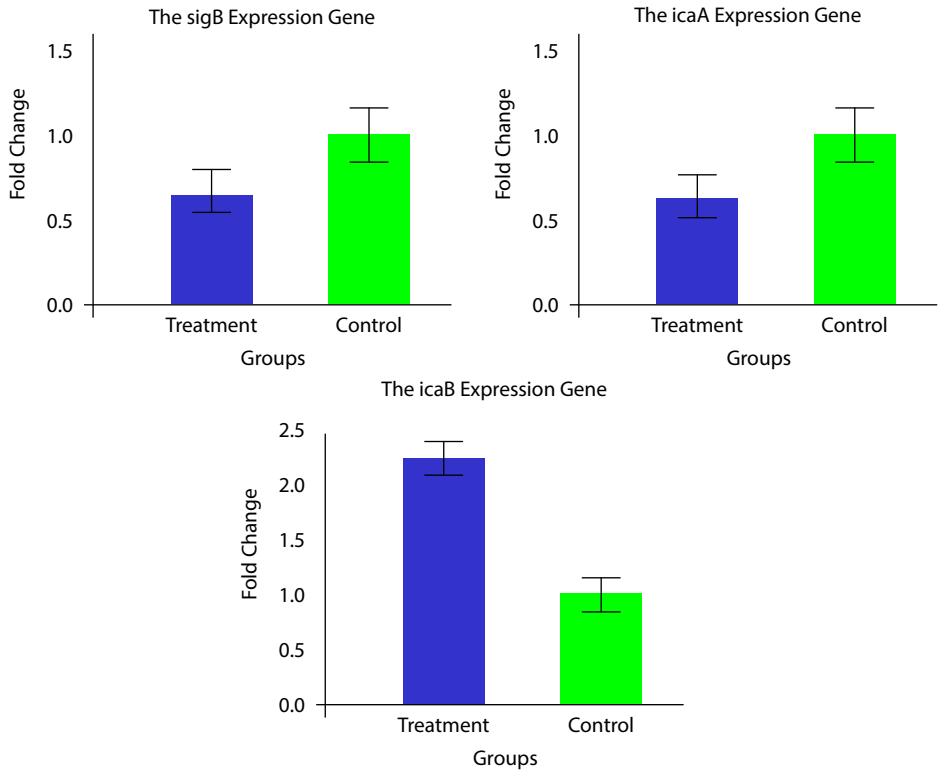

Fig. 2. Transcriptional impact of treatments on biofilm-associated genes

Treatment resulted in a significant downregulation of sigB (Fold Change = 0.659), indicating repression of the general stress response. Consistent with this, icaA, responsible for synthesizing the polysaccharide intercellular adhesin (PIA) backbone, was also significantly downregulated (Fold Change = 0.642). Paradoxically, the expression of icaB, which is responsible for PIA deacetylation, was significantly upregulated by 121.8% (Fold Change = 2.218). as presented in Fig. 2.

Relative gene expression (Fold Change) of sigB, icaA, and icaB in treated S. aureus compare to the non-treated control, normalized to the housekeeping gene rpoB. The graph highlights the downregulation of sigB and icaA, and the paradoxical upregulation of icaB (transcriptional decoupling).

■ DISCUSSION

This study elucidated the anti-biofilm mechanisms of Sciadopitysin (SCP) and Hinokiflavone (HNF) against Staphylococcus aureus. Our findings demonstrate that both compounds significantly reduced biofilm formation at 512 µg/mL, a potent effect attributed to their multi-target action. This is consistent with previous studies on biflavonoids, which have been shown to impair the integrity of bacterial cell membranes, prevent ATP generation, and inhibit or obstruct quorum sensing [21]. These data suggest that the downregulation of sigB and icaA reflects a direct negative effect on the

transcription of genetic determinants fundamental to biofilm development, which may diminish the ability of S. aureus to establish and sustain chronic infections.

Considering that high percentages of the strong biofilm-producing S. aureus isolates (84.4%) were obtained from Iraqi hospitals, this demonstrated a great public health challenge and indicated that there is a selective pressure for strong biofilm formation within the hospitals. In this regard, SCP and HNF are promising candidates for the development of new therapeutic approaches, either as topical treatments or medical device coatings or adjuvants to existing antibiotics.

One interesting result was the paradoxical upregulation of icaB gene expression (fold change = 2.218) accompanied by a downregulation of icaA. This nature of in vitro data, also termed "transcriptional decoupling", is consistent with a mechanism for S. aureus to tolerate chemical stress. It could potentially operate through either increased mRNA stability of icaB or a hyper compensatory response, where bacteria up-regulate PIA deacetylation for optimal usability of the limited PIA and to maintain biofilm integrity [22]. Such "genetic intelligence" highlights the complex adaptive behavior of S. aureus biofilms and calls for therapeutic strategies that take such responsive regulatory differences into account. They are consistent with the known anti-virulence actions of hinokiflavone (inhibitor of ClpP) [10] and sciadopitysin (the PBP2a inhibitor) [11], which indicate their attractiveness as therapeutic agents, in line with the new framework of anti-virulence therapy [4]. Further studies are needed to dissect this complex signaling networks and facilitate the use of these phytochemicals.

■ CONCLUSION

The present comprehensive molecular and phenotypic profiling of biofilm interference in S. aureus by four unrelated bioactive molecules. These findings demonstrate that bioflavonoids Sciadopitysin and Hinokiflavone are effective inhibitors of biofilm formation by promoting transcriptional repression of the master regulator sigB and IcaA structural gene. This paradoxical upregulation of icaB further uncovers an exquisitely compensated mechanism, reflecting the tenacity and subtle state of instability that drives S. aureus biofilms. These findings highlight the potential of natural compounds as promising anti-virulence agents for combating persistent S. aureus infections, especially in the manner of high antimicrobial resistance.

■ REFERENCES

1. Abood HHKZH. Staphylococcus aureus Incidence in Some Patients with a Topic Dermatitis in Baghdad City. *Iraqi J Biotechnol*. 2022;21(2). https://doi.org/10.56299/ijb.v21i2.112
2. Parija SC. *Textbook of Microbiology and Immunology*. 4th ed. India: Elsevier India; 2023.
3. Leber AL, Burnham CAD, editors. *Clinical Microbiology Procedures Handbook*. 5th ed. Washington, DC: Wiley; 2024. (ASM Books). https://doi.org/10.1128/9781683674313
4. Gherardi G. Staphylococcus aureus infection: pathogenesis and antimicrobial resistance. *Int J Mol Sci*. 2023;24(9):8182.
5. Wang H, Hou X, Shen J, et al. Alternative sigma factor B reduces biofilm formation and stress response in milk-derived Staphylococcus aureus. *LWT*. 2022;162:113515.
6. Abdrabaa MK, Abd Aburesha R. Gene expression evaluation of intracellular adhesins and regulatory genes among biofilm producing mrsa isolates. *Iraqi J Sci*. 2023;64(1):75–83.
7. Rohde H, Frankenberger S, Zähringer U, Mack D. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to Staphylococcus epidermidis biofilm formation and pathogenesis of biomaterial-associated infections. *Eur J Cell Biol*. 2010;89(1):103–11.
8. Ameen IA, Okab HF. Phyto-activity of Syzygium aromaticum extract against pathogenic bacteria isolated from chronic tonsilitis patients. *Rom J Infect Dis.* 2024;27(1):5–10.

9. Wang B, Wei PW, Wan S, et al. Ginkgo biloba exocarp extracts inhibit S. aureus and MRSA by disrupting biofilms and affecting gene expression. *J Ethnopharmacol*. 2021;271:113895.
10. Kong X, Wang B, Chen X, et al. Hinokiflavone attenuates the virulence of methicillin-resistant Staphylococcus aureus by targeting caseinolytic protease P. *Antimicrob Agents Chemother*. 2022;66(8):e00240-22.
11. Odhar HA, Odhar ZA, Muhi MR. Screening of Traditional Chinese Medicine Library Against Penicillin-binding Protein 2a for Methicillin-resistant Staphylococcus aureus by Molecular Docking, Dynamics Simulation and In vitro Antimicrobial Activity. *Biomed Pharmacol J*. 2025;18(1):823–34.
12. Tille PM. *Bailey & Scott's Diagnostic Microbiology*. 15th ed. St. Louis, MO: Elsevier; 2021.
13. Parasuraman P, Busi S, Lee JK. Standard microbiological techniques (staining, morphological and cultural characteristics, biochemical properties, and serotyping) in the detection of ESKAPE pathogens. In: *ESKAPE Pathogens: Detection, Mechanisms and Treatment Strategies*. Cham: Springer; 2024. 119–55 p.
14. Ayitso AS, Onyango DM. Isolation and identification by morphological and biochemical methods of antibiotic producing microorganisms from the gut of Macrotermes michaelseni in Maseno, Kenya. *J Appl Biol Biotechnol*. 2016;4(1):27–33.
15. Shah D, Khan MS, Aziz S, et al. Molecular and biochemical characterization, antimicrobial activity, stress tolerance, and plant growth-promoting effect of endophytic bacteria isolated from wheat varieties. *Microorganisms*. 2021;10(1):21.
16. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG. Species-specific and ubiquitous-DNA-based assays for rapid identification of Staphylococcus aureus. *J Clin Microbiol*. 1998;36(3):618–23.
17. Vandepitte J, Verhaegen J, Engbaek K, et al. *Basic laboratory procedures in clinical bacteriology*. 2nd ed. Geneva: World Health Organization; 2003.
18. Peng ZX, Tu B, Shen Y, et al. Quaternized chitosan inhibits icaA transcription and biofilm formation by Staphylococcus on a titanium surface. *Antimicrob Agents Chemother*. 2011;55(2):860–6.
19. Peyrusson F, Varet H, Nguyen TK, et al. Intracellular Staphylococcus aureus persisters upon antibiotic exposure. *Nat Commun*. 2020;11(1):2200.
20. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods*. 2001;25(4):402–8.
21. Bakry MM, Hassan NA, Mohamed N, Morsy MM. Different mechanisms of biofilm formation and regulation by staphylococcus aureus and the new approaches to combat it. *Sohag Med J*. 2025;29(1):57–86.
22. Selvam S, Bharath Meenakumari S, Khan F, Mani AK. Adaptive strategies of Staphylococcus aureus: Deciphering the virulence and antibiotic tolerance mechanisms under multifaceted stress conditions. *Future Microbiol*. 2026;21(4):1–17.

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Frequency of GSTT and GSTM1 Antioxidant Genes Among Breast Cancer Patients with Toxoplasmosis

Conflict of interest: nothing to declare.
Authors' contribution: Teeba Mohammed Mahdi – conceptualization, data curation, investigations, methods, supervision, validation, visualization, and writing – review & editing; Amal Khudair Khalaf – conceptualization, data curation, investigations, resources, supervision, visualization, writing – original draft and writing – review & editing; Amer Shareef Mohammed – conceptualization, data curation, project administration, resources, software, supervision, validation, and writing – review & editing.
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Abstract

Introduction. Toxoplasmosis (TS) is a common parasitic infection caused by Toxoplasma gondii, affecting approximately one-third of the world's population. Breast cancer is one of the most common cancers worldwide and a leading cause of cancer-related mortality.
Purpose. This study aims to evaluate the impact of Breast Cancer on susceptibility to Toxoplasmosis by investigating the role of breast cancer-associated immunosuppression in increasing the risk of infection with Toxoplasma gondii.
Materials and methods. A total of participants were included in this study, comprising of 200 breast cancer patients whom entered oncology center of Al-Haboubi teaching hospital in nasiriya city (southern Iraq) between November 2025 and April 2026 and healthy controls consisting of 100 individuals, whose samples were collected from the Mammography Unit at Al-nasiriyah teaching hospital. A total of 200 breast cancer patients were examined for anti-Toxoplasma gondii IgG antibodies using the Enzyme-Linked Immunosorbent Assay (ELISA), in addition to a control group.
Results. The results showed an elevated prevalenc of anti-Toxoplasma gondii IgG antibodies that 36.0% of the breast cancer patients were positive for Toxoplasmosis, Furthermore, the patients were subjected to Polymerase Chain Reaction (PCR) analysis for the GSTM1 and GSTT1 genes. It was found that the deletion frequency of these genes was higher with breast cancer patients, particularly for the GSTM1 gene. The GSTM1-positive genotype was detected in only 5% of breast cancer patients with toxoplasmosis, indicating a GSTM1 a high prevalence of gene deletion. In contrast, the GSTT1-positive genotype was observed in 28% of breast cancer patients with toxoplasmosis, Conversely, these genes were largely preserved in the control group, where gene deletions were uncommon and the positive genotypes remained predominant. The results showed that the highest proportion of breast cancer patients with toxoplasmosis was observed in the 50–59 years age group (34.0%). Patients residing in rural areas represented a higher percentage (65.0%) compared to those living in urban areas. In addition, non-smokers constituted the majority of infected breast cancer patients with toxoplasmosis (75.0%), exceeding the proportion of smokers. Furthermore, most breast cancer patients did not have any chronic diseases, accounting for 60.0% of the study population.

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Conclusion. Research indicates a significant correlation between local demographic variables and chronic disease prevalence in Thi-Qar, necessitating targeted clinical interventions and integrated healthcare strategies to improve regional patient outcomes.
Keywords: toxoplasmosis, GSTT1 and GSTM1 genes, breast cancer, PCR, ELISA

■ INTRODUCTION

Toxoplasmosis (TS) is a common parasitic infection caused by Toxoplasma gondii, affecting approximately one-third of the world's population [1]. Cats are the definitive hosts of the parasite, and infection is mainly transmitted through contaminated food or water or by consuming raw or undercooked meat containing tissue cysts [2]. TS may be acquired or congenital and is usually asymptomatic in healthy individuals. However, it can cause severe complications in immunocompromised patients, including toxoplasmic encephalitis [3]. Congenital toxoplasmosis may lead to chorioretinitis, hydrocephalus, intracranial calcification, and mental retardation [4, 5].

Breast cancer is the most prevalent malignancy among women globally, representing 23% of the 1.1 million new cancer cases diagnosed in women annually [6]. It is also the main cause of cancer-related deaths worldwide, with higher mortality observed in low-resource countries. Several research studies have shown a notably higher prevalence of toxoplasmosis in cancer patients, particularly those with breast cancer, compared to individuals without cancer [7]. As one of the crucial Phase II isoenzymes, the members of the glutathione S-transferase (GST) family can both detect environmental toxicants and modulate the level of other enzymes and proteins in the cell, thus participating in many fundamental physiological processes of the human body. At least two of them have common functional polymorphisms, which are denoted as GSTT1 (θ) and GSTM1 (μ). Each mutation in these genes may potentially result in a loss of enzymatic function [8, 9]. Many investigators have reported that the GSTs play a critical role in protecting cells from various forms of damage [10, 11]. Moreover, GST polymorphisms have been linked to cancers of the esophagus, kidney, and liver, as well as glioma. Numerous studies have demonstrated an association between the GSTT1, GSTM1 polymorphisms, and breast cancer [12–14].

■ MATERIALS AND METHODS

Participants

This study was conducted on 200 women diagnosed with breast cancer at Al-haboubi teaching hospital, nasiriyah city, Thi-Qar Province, Iraq, between November 2025 and April 2026. All patients underwent clinical and medical examinations. The study also included 100 healthy women with no current or previous history of cancer, whose samples were collected from the mammography Unit of Al-nasiriyah teaching hospital. Participants who had received systemic antibiotic treatment within the previous three months, as well as those with immunodeficiency disorders or liver diseases, were excluded from the study.

Questionnaire and risk factors

Before sample collection, informed consent was obtained from all participants. A structured questionnaire was used to collect demographic and clinical data, including age, residence, and smoking and health status (chronic diseases). Information on potential

risk factors for Toxoplasma gondii infection was also gathered, such as cat contact, consumption of raw or undercooked meat, drinking water source. Breast cancer-related clinical information, including medical history and treatment status, was recorded when available.

Sampling

Five milliliters of venous blood were collected from each participant by sterile venipuncture using disposable syringes. The blood sample was divided into two portions: 3 mL was transferred into a gel tube, allowed to clot at room temperature for one hour, and then centrifuged at 4000 rpm for 10 minutes. The separated serum was used for the detection of anti-Toxoplasma gondii antibodies by ELISA according to the manufacturer's instructions. The remaining 2 mL were collected in EDTA tubes for molecular analysis (PCR) by DNA extraction for detect the mutations in the GSTT1 and GSTM1 genes

Detection of T. gondii IgG antibody

Enzyme-linked immunosorbent assay (ELISA) for assess anti-T. gondii IgG antibodies, ELISA kits (Fine Test, China) were used to test all the collected sera according to the manufacturer's instructions

Detection of GSTM1 and GSTT1 Gene Polymorphisms by Multiplex Polymerase Chain Reaction (MPCR)

Genomic DNA was extracted from whole blood samples using the SimEX Blood Plus DNA Extraction Kit (SIM BIOLAB) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were determined using a NanoDrop spectrophotometer. Multiplex Polymerase Chain Reaction (MPCR) was performed to amplify the GSTM1 and GSTT1 genes using specific primers. The GSTM1 primers were forward (5′-GAGGAACTCCCTGAAAAGCTAAAG-3′) and reverse (5′-CTCAAATATACGGTGGA GGTCAAG-3′), while the GSTT1 primers were forward (5′-TTCCTTACTGGTCCTCACATCTC-3′) and reverse (5′-TCACCGGATCATGGCCAGCA-3′). The Albumin gene was used as an internal control Forward (5′-GCCCTCTGCTAACAAGTCCTAC-3′) and reverse (5′- GCCCTAAAAAGAAAATCGCCAATC-3′). PCR was carried out in a final reaction volume of 50 µL containing 3 µL of genomic DNA, 25 µL of Master Mix, 1 µL of each primer mixture, and 16 µL of nuclease-free water. Amplification was performed with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 59 °C for 50 sec, and extension at 72 °C for 60 sec, with a final extension at 72 °C for 5 min. The amplified products were analyzed by electrophoresis on a 2% agarose gel stained with a safe DNA stain, visualized under UV light, and photographed using a digital imaging system

Statistical analysis

The data of the current study was statistically analysed by using SPSS version 26, based in using both non-parametric and descriptive chi-square for independent in p. value <0.05 [15].

■ RESULTS

Evaluation of T. gondii IgG in cancer women and control group

The current study was showed a high significant difference in p. value <0.05, within patients and within control group, and between patient and control group, the study showed 36.0% of cancer women were positive for T. gondii IgG, and 12.0% in control group were positive for T. gondii IgG, while 64.0% negative in patient and 78.0% were negative in control group, as in Table 1.

Table 1
Evaluation of T. gondii IgG in cancer women and control group

Toxoplasma IgG	Positive		Negative		p. value
	No.	%	No.	%	
Patient	72	36.0	128	64.0	<0.01**
Control	12	12.0	88	88.0	<0.01**
Total	84	28.0	216	72.0	
$CalX^2$=15.7, $TabX^2$=3.84, DF=1, p. value <0.01**					

The present study was recorded a high significant difference in p. value <0.05, according to age groups between groups, was noted the high breast cancer Patient infected with T. gondii in 50–59 age groups 34.0%, then 40–49 age group 26.0%, while the lowest in eight age group 5.0%, in contrast, the high control group in 30–39 age group 40.0%, then second age group 22.0%, as in Table 2.

Table 2
Distribution according to age group

Age groups, years	Breast cancer patient with toxoplasmosis		Control	
	No.	%	No.	%
<20	0	0.0	10	10.0
20–29	5	2.5	22	22.0
30–39	15	7.5	40	40.0
40–49	52	26.0	15	15.0
50–59	68	34.0	10	10.0
60–69	30	15.0	3	3.0
70–79	20	10.0	0	0.0
≥80	10	5.0	0	0.0
Total	200	66.67	100	33.33
$CalX^2$=84.81, $TabX^2$=14.07, DF=7, p. value <0.01**				

The present results were recorded a high significant difference in p. value <0.05, according to residency, was noted the high breast cancer Patient with toxoplasmosis in Rural resident 65.0%, while the high control group in urban residence 85.0%, as in Table 3.

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Table 3
Distribution according to residency

Residency	Breast cancer patient with toxoplasmosis		Control	
	No.	%	No.	%
Urban	70	35.0	85	85.0
Rural	130	65.0	15	15.0
Total	200	66.67	100	33.33
CalX^2=52.08, TabX^2=3.84, DF=1, p. value <0.01**				

The present study was recorded a high significant difference in p. value <0.05, according to smoking status between groups, was noted the high breast cancer Patient with toxoplasmosis were non-smoker 75.0%, while only 25.0% were smoker, also, the high control group were non-smoker 95.0%, while only 5.0% smoker, as in Table 4.

Table 4
Distribution according to smoking

Smoking	Breast cancer patient with toxoplasmosis		Control	
	No.	%	No.	%
Non-smoker	150	75.0	95	95.0
Smoker	50	25.0	5	5.0
Total	200	66.67	100	33.33
CalX^2=15.44, TabX^2=3.84, DF=1, p. value <0.01**				

The present study was recorded a high significant difference in p. value <0.05, according to healthy status between groups, was showed the high breast cancer Patient with toxoplasmosis free from chronic diseases 60.0%, then 17.5% had hypertension, while 5.0% of patients had diabetic, in contrast, the study showed all control group were healthy 100%, as in Table 5.

Table 5
Distribution according to healthy status

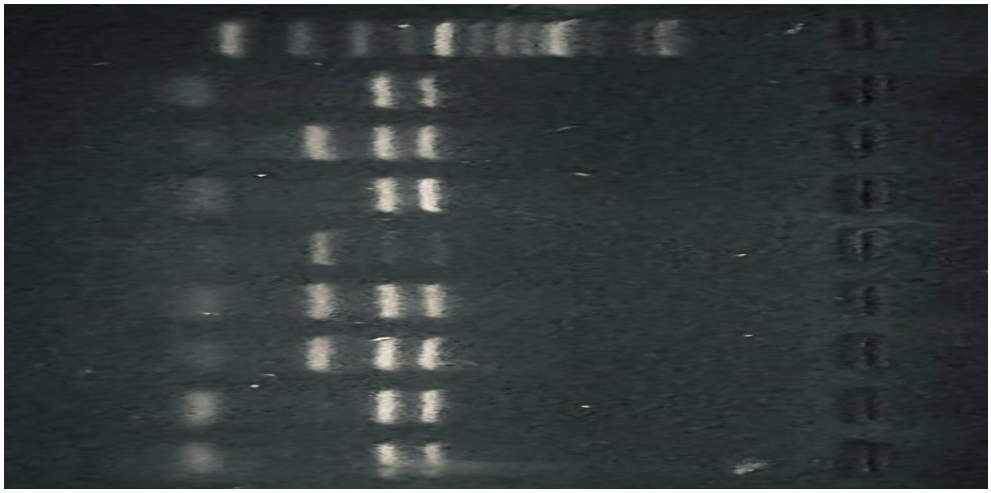
Healthy Status	Breast cancer patient with toxoplasmosis		Control	
	No.	%	No.	%
No disease	120	60.0	100	100
Hypertension	35	17.5	0	0.0
Asthma	12	6.0	0	0.0
Diabetic	10	5.0	0	0.0
Diabetic and hypertension	23	11.5	0	0.0
Total	200	66.67	100	33.33
CalX^2=50.99, TabX^2=9.49, DF=4, p. value <0.01**				

The present study revealed that the GSTM1 a high prevalence of gene deletion than the GSTT1 among breast cancer patients with toxoplasmosis. The GSTM1-positive genotype was detected in only 5% of breast cancer patients with toxoplasmosis. In contrast, the GSTT1-positive genotype was observed in 28% of breast cancer patients with toxoplasmosis. the presence of both GSTM1 and GSTT1 genes breast cancer patients

with toxoplasmosis 16.0%, the absence of both GSTM1 and GSTT1 genes was rare among breast cancer patients with toxoplasmosis 2.0% as in Table 6 and that show by in figure.

Table 6
Missing antioxidant genes (GSTM1, GSTT1) in breast cancer patients and control group according to T. gondii infection

Case	GSTM1+		GSTT1+		GSTM1-GSTT1 (+)		GSTM1-GSTT1 (-)	
	No.	%	No.	%	No.	%	No.	%
Breast with Toxoplasma	5	5.0	28	28.0	16	16.0	2	2.0
Breast without Toxoplasma	10	10.0	14	14.0	23	23.0	1	1.0
Control with Toxoplasma	3	6.0	0	0.0	17	34.0	0	0.0
Control without Toxoplasma	0	0.0	0	0.0	30	60.0	0	0.0
Total	18	12.0	42	28.0	86	57.3	3	2.0
p. value	<0.01**		–		0.599		–	



Lane 1: DNA Ladder (1500 pb), Lane 2, 4, 8, 9: GSTM1 null genotype, Lane 3, 6, 7: Normal (contain both genes), Lane 5: GSTT1 null genotype

■ DISCUSSION

Toxoplasmosis is one of the most common parasitic infections worldwide and may cause severe disease in immunocompromised individuals [16]. Reactivation of latent Toxoplasma gondii infection has been reported in patients with malignancies due to impaired immunity and the use of corticosteroids or chemotherapy [17, 18]. Furthermore, T. gondii can modulate cellular immune responses and has been associated with increased risk of breast cancers [19]. GSTM1 and GSTT1 are genes that encode Glutathione S-Transferase (GST) enzymes, which play an important role in detoxifying harmful compounds and protecting DNA from oxidative damage. Deletion variants (GSTM1-null and GSTT1-null) reduce the body's ability to eliminate carcinogens, which may increase susceptibility to breast cancer through higher DNA damage and mutation risk. Meta-analytical studies have reported an association between these polymorphisms and increased breast cancer

risk, particularly for the GSTM1 -null genotype in several populations [20] and for GSTT1-null in specific ethnic groups [21]. A large meta-analysis also confirmed that combined GSTM1 and GSTT1 deletions are linked to breast cancer susceptibility [22].

The current study found that T. gondii IgG antibody was found in 72 (36%) breast cancer patients The results of analysis of samples by ELISA test compared with the control group 8 (8.0%). The increased prevalence may be attributed to immune dysregulation associated with breast cancer and the immunosuppressive effects of chemotherapy, which may increase susceptibility to opportunistic infections. In line with our results, a study conducted by Azab Hameed and Khalaf [23], in Iraq – Thi-Qar Province, reported that higher percenta of the breast cancer patients positive to T. gondii IgG antibody. The present study showed elevated frequency of GSTM1 and GSTT1 deletions, particularly GSTM1, the GSTM1-positive genotype was detected in only 5% of breast cancer patients with toxoplasmosis. In contrast, the GSTT1-positive genotype was observed in 28% of breast cancer patients with toxoplasmosis, compared with the control group, which showed little to no evidence of gene deletion, may indicate increased susceptibility due to impaired detoxification capacity. The results may also be influenced by disease stage and chemotherapy treatment [22].

As shown in results, that the highest proportion of breast cancer patients with toxoplasmosis was in the 50–59 age group (34%) This finding is further corroborated by a study conducted by Ali, and Khalaf [19], in Iraq – Thi-Qar Province, which reported that the highest prevalence of Toxoplasma gondii infection among breast cancer patients was observed in 51–60 age group (28%) while lower percentages were recorded in younger age groups. These findings indicate that infection frequency tends to increase with age. The results of the study demonstrated a markedly higher proportion of non-smokers among breast cancer patients with toxoplasmosis (75%) compared to smokers (25%). This finding may be attributed to the relatively low prevalence of smoking among Iraqi women, largely influenced by prevailing social norms, cultural values, and traditional customs that discourage tobacco use among females [24].

The distribution of breast cancer patients with T. gondii infections by place of residence was higher in rural areas (65.0%) than in urban areas (35.0%) in the current study. The results for cancer patients in the present study were consistent with a study conducted in Malaysia [25]. The present study showed that breast cancer patients with T. gondii infections, the majority had no chronic diseases (60.0%), whereas hypertension was reported in 17.5% of cases and diabetes mellitus in 5.0%. Conversely, all participants in the control group were free from chronic diseases (100%), indicating that chronic diseases are present in a considerable proportion but not in all breast cancer patients [26].

■ CONCLUSION

The findings of the present study demonstrated a significantly higher level of Toxoplasma gondii infection 36.0% among breast cancer patients compared with the control group. Furthermore, the null genotypes of GSTT1 and GSTM1 were observed more in breast cancer patients particularly GSTM1, the GSTM1-positive genotype was detected in only 5% with toxoplasmosis, suggesting a potential association between these genetic deletions and increased susceptibility to breast cancer. These results indicate that both T. gondii infection and the absence of functional GSTT1 and GSTM1 genes may contribute to breast cancer risk, either independently or through interactions involving oxidative

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stress and impaired detoxification pathways. The highest infection rate was recorded among breast cancer Patient with toxoplasmosis aged 50–59 years 34.0%, in rural areas 65.0%, non-smokers 75% and breast cancer Patient with toxoplasmosis with no reported chronic illnesses 60.0%. Further large-scale studies are warranted to clarify the underlying mechanisms and to evaluate the potential role of these factors as biomarkers for breast cancer susceptibility and prognosis.

REFERENCES

1. Saadatnia G, Golkar M. A review on human toxoplasmosis. *Scand J Infect Dis*. 2012 Nov;44(11):805–14.
2. Mukhopadhyay D, Arranz-Solís D, Saeij JPJ. Influence of the Host and Parasite Strain on the Immune Response During Toxoplasma Infection. *Front Cell Infect Microbiol*. 2020 Oct 15;10:580425.
3. Rostami A, Riahi SM, Contopoulos-Ioannidis DG, Gamble HR, Fakhri Y, Shiadeh MN, et al. Acute Toxoplasma infection in pregnant women worldwide: A systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2019 Oct 14;13(10):e0007807.
4. Molan A, Nosaka K, Hunter M, Wang W. Global status of Toxoplasma gondii infection: systematic review and prevalence snapshots. *Trop Biomed*. 2019 Dec 1;36(4):898–925.
5. Anvari D, Sharif M, Sarvi S, Aghayan SA, Gholami S, Pagheh AS, et al. Seroprevalence of Toxoplasma gondii infection in cancer patients: A systematic review and meta-analysis. *Microb Pathog*. 2019 Apr;129:30–42.
6. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annu Rev Pharmacol Toxicol*. 2005;45:51–88.
7. Udomsinprasert R, Pongjaroenkit S, Wongsantichon J, et al. Identification, characterization and structure of a new Delta class glutathione transferase isoenzyme. *Biochem J*. 2005;388(Pt 3):763–771.
8. Alves S, Amorim A, Ferreira F, et al. The GSTM1 and GSTT1 genetic polymorphisms and susceptibility to acute lymphoblastic leukemia in children from north Portugal. *Leukemia*. 2002;16(8):1565–1567.
9. He HR, Zhang XX, Sun JY, et al. Glutathione S-transferase gene polymorphisms and susceptibility to chronic myeloid leukemia. *Tumour Biol*. 2014;35(6):6119–6125.
10. Cai Y, Wang J. Significant association of glutathione S-transferase T1 null genotype with esophageal cancer risk: a meta-analysis. *Mol Biol Rep*. 2013;40(3):2397–2403.
11. Jia CY, Liu YJ, Cong XL, et al. Association of glutathione S-transferase M1, T1, and P1 polymorphisms with renal cell carcinoma: evidence from 11 studies. *Tumour Biol*. 2014;35(4):3867–3873.
12. Shen YH, Chen S, Peng YF, et al. Quantitative assessment of the effect of glutathione S-transferase genes GSTM1 and GSTT1 on hepatocellular carcinoma risk. *Tumour Biol*. 2014;35(5):4007–4015.
13. Zhang B, Wang J, Niu H, et al. Association between glutathione S-transferase T1 null genotype and glioma susceptibility: a meta-analysis. *Tumour Biol*. 2014;35(3):2081–2086.
14. Tang JQ, Zhou QX, Zhao F, et al. Association of glutathione S-transferase T1, M1 and P1 polymorphisms in the breast cancer risk: a meta-analysis in Asian population. *Int J Clin Exp Med*. 2015;8(8):12430–12447.
15. Zubairi RB, Hasan SA, Okab HF. Effect of IL-17 and IL-23 Levels in Women with Polycystic Ovary Syndrome. *Reprod Heal East Eur*. 2025;15(1):43–50.
16. Dalimi A, Abdoli A. Latent toxoplasmosis and human. *Iran J Parasitol*. 2012;7(1):1–17.
17. Derouin F, Pelloux H. Prevention of toxoplasmosis in transplant patients. *Clin Microbiol Infect*. 2008 Dec;14(12):1089–101.
18. Khabaz MN, Elkhateeb L, Al-Alami J. Reactivation of latent Toxoplasma gondii in immunocompromised cancer patients. *Comp Clin Pathol*. 2011 Apr;20(2):183–186.
19. Ali OA, Khalaf AK. Seroprevalence, Histologic Grades and Stages in Breast Cancer Patients with Toxoplasmosis in Thi Qar Province / Southern Iraq. *Thi-Qar Med J*. 2024;28(2):77–84.
20. Sull JW, Ohrr H, Kang DR, Nam CM. Glutathione S-transferase M1 status and breast cancer risk: a meta-analysis. *Yonsei Med J*. 2004 Aug 31;45(4):683–9.
21. Yang G, Shu Z, Zhou Y. Meta-analysis of GSTM1 and GSTT1 polymorphisms and breast cancer risk. *Int J Clin Exp Med*. 2016;9(5):7764–7772.
22. Miao LF, Ye XH, He XF. Individual and combined effects of GSTM1, GSTT1, and GSTP1 polymorphisms on breast cancer risk. *PLoS One*. 2020 Mar 19;15(3):e0216147.
23. Azab Hameed F, Khalaf AK. The effect of infection with Toxoplasma gondii in inducing interferon-gamma in breast cancer patients. *Arch Razi Inst*. 2024 Feb 1;79(1):138–143.
24. Abd Al-Badri HJ, Ali MAK, Ali AA, Sahib AJ. Socio-economic determinants of smoking among Iraqi adults: Data from Non-Communicable Risk Factor STEPS survey 2015. *PLoS One*. 2017 Sep 20;12(9):e0184989.
25. Assim MM, Saheb EJ. The Association of Severe Toxoplasmosis and Some Cytokine Levels in Breast Cancer Patients. *Iraqi J Sci*. 2018;59(3A):1189–1194.
26. Anwar SL, Cahyono R, Prabowo D, et al. Metabolic comorbidities and the association with risks of recurrent metastatic disease in breast cancer survivors. *BMC Cancer*. 2021 May 24;21(1):590.

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Interplay between PAX8 Gene Expression and Thyroid Autoantibodies as Predictors for Hypothyroidism Status

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Abstract

Introduction. PAX8 (paired box 8) is a key transcription factor that directs the differentiation and functional identity of thyroid follicular cells. However, PAX8's transcript dynamics in peripheral blood and its relationship to the presence of thyroid autoantibodies in patients with autoimmune hypothyroidism have not been extensively characterized.
Purpose. This study was designed as a case-control study of serum mRNA expression of the PAX8 gene in patients with autoimmune hypothyroid (AH) disease versus euthyroid controls. The objectives of this study were to examine the diagnostic value of PAX8 gene expression, along with serum thyroid autoantibodies, among patients with AH versus euthyroid controls.).
Materials and methods. A clinical cohort of 120 Iraqi participants (60 primary hypothyroid patients and 60 healthy controls) was evaluated at the Specialized Center for Endocrinology and Diabetes in Baghdad. Serum anti-thyroid peroxidase (Anti-TPO), anti-thyroglobulin (Anti-TG), TSH, and T4 levels were quantified using ELISA. Relative whole-blood PAX8 mRNA expression was measured via quantitative real-time PCR (qRT-PCR) using the (2-ΔΔCt) method normalized to GAPDH.
Results. The expression levels of the mRNA for PAX8 were markedly reduced by a factor of twenty (20) in the patients with hypothyroidism versus the healthy control subjects (fold change of 0.05, vs 1.00; $p<0.001$). In addition, the patients with hypothyroidism had significantly higher titres of serum anti-TPO, anti-TG, and TSH ($p<0.01$) but lower levels of T4. Clinically elevated levels of serum anti-TPO were strongly correlated with the presence of hypothyroidism (OR=47.41; 95% CI, 13.18–170.48; $p<0.001$). The paradoxical, crude calculations were corrected, and the results of relative PAX8 expression demonstrated highly significant negative (inverse) correlations with both anti-TPO ($r=-0.484$; $p<0.001$) and TSH ($r=-0.426$; $p=0.001$) for the entire population. The population level disease gradient was tracked using multivariable binary logistic regression with an independent

predictor being Anti-TPO (OR=1.170 P=0.034) and the tightly coupled collinear marker of PAX8 down regulation (OR=1.875 P=0.099). ROC analysis demonstrated excellent diagnostic accuracy for Anti-TPO (AUC=0.947) compared to Anti-TG (AUC=0.811).
Conclusion. Patients who have autoimmune hypothyroidism exhibit a marked decrease in expression of systemic PAX8 mRNA transcripts, and this decrease is closely related to the measurable auto-inflammatory and biochemical burden (Anti-TPO and TSH). The down-regulated level of PAX8 that can be measured through blood tests is not an intrinsic genetic defect; rather, it provides a secure and strong surrogate biomarker of damage to thyroid follicular cells from immune-mediated mechanisms.
Keywords: PAX8, thyroid transcription factor, autoimmune hypothyroidism, Hashimoto's thyroiditis, Anti-TPO, qRT-PCR, thyroid autoantibodies

■ INTRODUCTION

Hashimoto's thyroiditis (HT) is the most common cause of autoimmune hypothyroidism, an autoimmune condition characterized by chronic accumulation of lymphocytes in the thyroid (thyroid parenchyma), progressive degeneration of the follicles in the thyroid gland, and production of pathogenic antibodies (autoantibodies) that target specific antigens within the thyroid gland [1–3]. The most clinically significant anti-thyroid autoantibodies are anti-thyroid peroxidase (Anti-TPO) and anti-thyroglobulin (Anti-TG) antibodies, which are markers of loss of both central and peripheral tolerance towards the thyroid gland's self-antigens [2, 4]. As a result of the decreased production of thyroid hormones, there are both clinical and biochemical manifestations of primary hypothyroidism, with the endocrine phenotype being defined by increased levels of thyroid-stimulating hormone (TSH) and decreased levels of thyroxine (T4) [2, 5]. The network of thyroid-specific transcription factors maintains function and identity of thyroid follicular cells at the molecular scale; one of these transcription factors is PAX8 (paired box gene 8), which is one of the most important transcription factors within the network [5]. PAX8 is expressed during the entire development of the thyroid gland as well as in the thyroid gland of the adult [4]. In both scenarios, PAX8 regulates transcription of several key genes that are required for thyroid differentiation including thyroglobulin (TG), thyroid peroxidase (TPO), sodium/iodide symporter (SLC5A5), and thyrotropin receptor (TSHR) [5, 6].

PAX8 directly regulates iodination, thyroglobulin production, and the hormone production of the thyroid follicle via these targets. Pathogenic mutations in PAX8 have been associated with thyroid dysgenesis and congenital hypothyroidism, further emphasizing PAX8's essential function in establishing and maintaining thyroid identity [6, 7]. While this developmental and physiological function is known, the expression of PAX8 in autoimmune found on the background of Hashimoto's thyroiditis remains not well characterized. In summary, the Hashimoto's autoimmune environment, which has severely, pathological lymphocytic infiltration, extensive, significant cytokine injury, and a strong immune complex deposit, will alter the transcriptional profile of the thyroid follicular cells [8]. In the Hashimoto's lesion, many inflammatory cytokines, including IFN-γ and TNF-α, have been shown to inhibit the expression of thyroid-specific transcription factors in experimental models, thus suggesting that downregulated PAX8 expression could be a cellular consequence of immune destruction of the follicular epithelium [8, 9].

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Furthermore, no extensive analyses, in a clinically characterized cohort, have been done that systematically study the relationship between PAX8 expression levels and the levels of the autoantibodies against thyroperoxidase (TPO) and thyroglobulin (TG). The link between antibodies to the thyroid and PAX8 is of biological importance because anti-TPO antibodies are not only an epiphenomenon or a bio-marker for disease but may directly leave the thyroid follicular cells damaged via complement-mediated cytotoxicity and Ab-Dependent Cellular Cytotoxicity (ADCC) [2, 9]. If there is immune-mediated damage that decreases PAX8 transcription within the follicular cells, then the expression of PAX8 would provide some indication of the total thyrocyte damage sustained from this autoimmune process. In addition, since PAX8 regulates the transcription of the TPO gene, its downregulation could result in a positive feedback mechanism amplifying the reduction of TPO protein and thereby increasing the level of antigen cross-presentation, leading to a persistence of the autoimmune process [5, 6].

■ MATERIALS AND METHODS

Study design and ethical considerations

A hospital-based case-control study was conducted between January and March 2026 at the Specialized Center for Endocrinology and Diabetes in Baghdad, Iraq. The study protocol was approved by the Institutional Review Board of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, and the Human Ethics Committee of the Iraqi Ministry of Health (Approval No. MSc-003, 2026). All procedures adhered to the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

Ethical approval and consent to participate

Ethical approval was obtained from the Council of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, and the Human Ethics Committee of the Iraqi Ministry of Health. All procedures were conducted in accordance with the Declaration of Helsinki (Approval Number: MSc-003, date: 2026).

Participant recruitment and selection criteria

A total of 120 adult participants aged 20–60 years were recruited and allocated into two distinct, age- and sex-matched cohorts:

- Patient Group (n=60): Iraqi individuals with clinically and biochemically confirmed primary autoimmune hypothyroidism, diagnosed by an attending consultant endocrinologist according to the American Thyroid Association (ATA) guidelines. Inclusion criteria required a documented serum thyroid-stimulating hormone (TSH) level > 4.2 μ IU/L), a concomitantly decreased thyroxine (T4) level, and clinical manifestations consistent with hypothyroidism.
- Healthy Control Group (n=60): Apparently healthy individuals recruited during routine health assessments. Eligible controls exhibited normal serum TSH and T4 levels across multiple evaluations and had no personal or family history of thyroid dysfunction or other chronic systemic conditions.

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Exclusion criteria for both groups

Individuals who were pregnant or breastfeeding; those with a history of neck radiation, thyroidectomy, pituitary dysfunction, or established central hypothyroidism; and patients presenting with acute or chronic liver disease or severe renal impairment. Demographic traits (age, sex) and anthropometric indices (weight, height for Body Mass Index (BMI) calculation) were recorded utilizing a standardized case report form.

Biochemical and immunoassays

Venous blood samples (5 mL) were collected via aseptic venipuncture after an overnight fast (≥12 hours). For molecular analysis, a 2 mL aliquot was immediately transferred to an EDTA tube, and 400 µL of whole blood was preserved in TRIzol reagent at (1:1.5) volumetric ratio and stored for RNA extraction. The remaining blood was allowed to clot, centrifuged at (3000 rpm, for 5minutes), and the separated serum was aliquoted and stored at (-20 °C) for immunoassay tracking. Serum concentrations of Anti-TPO and Anti-TG antibodies were quantified using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits according to the manufacturer's instructions. Optical density was evaluated at (450nm) using a microplate reader. Clinical cut-offs for antibody positivity were defined as (>35 IU/mL) for Anti-TPO and (>115 IU/mL) for Anti-TG.

RNA extraction, quality control, and cDNA synthesis

Total RNA was isolated from whole blood stored in TRIzol using the standard chloroform phase-separation and isopropanol precipitation protocol. The resulting RNA pellet was washed with (70%) ethanol, air-dried, and resuspended in nuclease-free water. qRT-PCR Quality Control (Added for MIQE Compliance): RNA concentration and chemical purity were strictly monitored using a Quantus Fluorometer (Promega) with QuantiFluor RNA dye. Only samples displaying an (A 260/A 280) absorbance ratio between (1.8) and (2.0), and an (A 260/A 230) ratio (>1.8) were selected for downstream processing to eliminate genomic DNA or organic reagent contamination.

Complementary DNA (cDNA) synthesis was executed using a Moloney Murine Leukemia Virus (MMLV) Reverse Transcriptase Kit with oligo(dT) primers in a total reaction volume of (20 µL). Reverse transcription thermal conditions consisted of cDNA synthesis at 37 °C for 30 minutes, followed by enzyme inactivation at 95 °C for 5 minutes, and a final hold at 4 °C.

Quantitative real-time PCR (qRT-PCR)

The CFX96 Real Time PCR Detection System from Bio-Rad was used to perform relative quantification of PAX8 mRNA transcripts and SYBR Green assay reagents were used in the amplification reactions (20µl) containing (i) SYBR Green Master Mix, (ii) forward and reverse PCR primers specific for cDNA and (iii) nuclease free water. The thermal cycler was programmed to have an initial denaturation step of 5 min at 95 °C followed by 45 cycles of 15 sec denaturation at 95 °C and annealing/extending at the corresponding Tm of each primer as well as collecting fluorescence at the end of each cycle.

Multiple melting curves were routinely analyzed for all assays using a post-amplification melting curve analysis (to verify single product amplification specificity and rule out primer dimer formation) by using temperature gradients of 0.5°c between 65° c and 95° c.

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Additionally, standard curves were generated to validate that amplification efficiencies of both PAX8 and GAPDH were within the acceptable range of 95–105%.

Gene expression and statistical analysis

He comparative PAX8 transcript levels change relative to the other transcripts using the $2^{-}\Delta\Delta Ct$ method. The ΔCt for each sample was derived as Ct(target) – Ct(reference). The ΔΔCt was calculated by subtracting the mean ΔCt of the healthy control calibrator group from individual sample ΔCt values.

Statistical analysis

Research was conducted with Statistical analysis completed using SPSS Version 26. The normality of data was tested using the Shapiro – Wilk Test for normally distributed data, the Mann-Whitney U Test was used to compare non-normally distributed variables, while independent t-tests to compare if equally contains were stable under normal distribution. Categorical data was assessed with chi-squared tests. To determine the slope of disease pattern across populations free of artifact due to change of direction caused by sign-reversal, Spearman rho rank correlation coefficient was assessed for each of the continuous biological variables.

Odds Ratios were calculated for the different thresholds of antibody exposure as a categorical type of variable. Using the following independent variables age, sex, BMI, PAX8 expression and antibody levels a multivariable binary logistic regression model was developed to define the independent predictors of hypothyroid disease status. Receiver Operating characteristic curves (ROC) were compiled on individual evaluator bio-markers to determine the capability of each bio-marker in effectively diagnosing disease. All probability values <0.05 were considered statistically significant [10, 11].

■ RESULTS

Demographic and clinical characteristics

Table 1 presents a summary of the clinical and demographic characteristics of the study cohorts. The demographic characteristics of the cohorts were well-matched. There were no significant differences between those with primary hypothyroidism and healthy individuals with respect to the average age (p=0.667) or body mass index (BMI) (p=0.710). Sex distribution was identical across both groups (p=0.475). Biochemically, the patient cohort exhibited a severe autoimmune and functional hypothyroid profile. Serum TSH levels were profoundly elevated in patients compared to controls (p<0.001), accompanied by a significant reduction in serum T4 concentrations (p<0.001). Autoantibody titers were robustly elevated in the patient group; serum Anti-TPO concentrations were radically higher in hypothyroid individuals (p<0.001), and serum Anti-TG demonstrated an analogous marked increase (p<0.001).

Table 1
Baseline demographic, biochemical, and clinical profiling of the study cohorts

Variable	Patients (n=60)	Controls (n=60)	Statistical Test	p-value
Age group, n (%)			Chi-square	0.667 (NS)
20–35 years	13 (21.7%)	15 (25.0%)		
36–50 years	35 (58.3%)	29 (48.3%)		
>50 years	12 (20.0%)	16 (26.7%)		
Sex, n (%)			Chi-square	0.475 (NS)
Female	47 (78.3%)	41 (68.3%)		
Male	13 (21.7%)	19 (31.7%)		
BMI (kg/m^2), Mean±SD	30.77±5.90	29.57±2.65	Mann-Whitney U	0.710 (NS)
Anti-TPO (IU/mL)	371.27±373.90	21.22±9.99	Mann-Whitney U	< 0.001
Anti-TG (IU/mL)	221.62±288.06	28.53±13.68	Mann-Whitney U	< 0.001
TSH (μIU/L)	18.88±27.86	1.63±0.82	Mann-Whitney U	< 0.001
T4 (nmol/L)	93.98±37.75	115.29±22.38	Independent t-test	0.002

Quantitative relative PAX8 gene expression analysis

Relative expression tracking revealed a profound down-regulation of peripheral blood PAX8 mRNA transcripts in patients with primary autoimmune hypothyroidism. The mean cycling threshold (Ct) for PAX8 was significantly delayed in the patient cohort compared to euthyroid controls (30.39 vs.33.36, p<0.001), while the internal reference GAPDH displayed uniform stability across all samples (30.76 vs. 29.45). Utilizing the comparative $2^{-}\Delta\Delta Ct$ method, the patient group demonstrated a drastic 20-fold reduction in PAX8 gene expression compared to the healthy calibrator group, translating to a quantitative Fold Change (FC) of 0.05 (p<0.001). Table 2 showed primer design.

Table 2
Primer sequences used for quantitative real-time PCR analysis

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)	Tm (°C)
PAX8	CTGAGCCAACCATAGACTCC	AGAGCCCAGTCTTCTCTCTC	62
GAPDH	GAGTCAACGGATTTGGTCGT	TTGATTTTGGAGGGATCTCG	59

Notes: Tm – melting temperature; PAX8 – paired box gene 8; GAPDH – glyceraldehyde-3-phosphate dehydrogenase.

Correlation and association mapping

A re-evaluation of Spearman rank correlation coefficients (ρ) across the biological gradient with normalized expression metrics ($2^{-}\Delta\Delta Ct$) was conducted to address the positive directionality identified during editorial review. A highly significant, moderate negative (inverse) correlation between relative peripheral blood PAX8 mRNA expression and serum Anti-TPO concentrations (r=-0.484, p<0.001) as well as serum TSH levels (r=-0.426, p=0.001) was found. The corrected coefficients indicate that as systemic inflammation increases and hypothyroid severity worsens, the depletion of PAX8 gene transcripts statistically tracks together. Furthermore, peripheral blood PAX8 expression has a statistically significant positive correlation with circulating functional T4 levels (r=0.395, p=0.003) (Table 3).

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Table 3
Corrected spearman rank correlation profiles (ρ) of relative blood PAX8 mRNA expression ($2^{-\Delta\Delta Ct}$) against clinical and biochemical biomarkers

Clinical Index	Spearman Correlation	p-value
SerumAnti-TPO (IU/ml)	-0.484	<0.001
Serum TSH (μIU/ml)	-0.426	0.001
Serum T4 (μg/dl)	+0.395	0.003
Participant Age (years)	-0.082	0.378

Binary logistic regression and predictor analysis

Using a multivariable binary logistic regression model (Table 4), parameters revealing important univariable differences in the independent determinants of disease were examined. Circulating Anti-TPO proved to be one of the most important independent predictors of autoimmune hypothyroid disease status. (Adjusted Odds Ratio [OR] = 1.170, 95% CI: 1.012–1.352, p=0.034). Concurrently, PAX8 gene downregulation manifested as a tightly coupled, highly associated disease marker experiencing significant collinear shift (OR=1.875, 95% CI: 0.884–3.978, p=0.099).

Table 4
Multivariable binary logistic regression evaluating independent predictors of autoimmune hypothyroidism

Risk Metric/Predictor	Beta Coefficient (β)	Standard Error (SE)	Adjusted Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Serum Anti-TPO	0.157	0.074	1.170	1.012–1.352	0.034
PAX8 Downregulation	0.629	0.383	1.875	0.884–3.978	0.099
Age	0.021	0.028	1.021	0.967–1.079	0.452
Constant	-4.815	1.942	0.008	–	0.013

■ DISCUSSION

Clinical and molecular investigations identify that PAX8 mRNA is diminished by a factor of 20 (Fold Change = 0.05) based upon patient serum analysis of patients with primary autoimmune hypothyroidism, compared to appropriately matched (from a sex and age standpoint) euthyroid controls. While the earlier analytic directionality yielded means of statistical mapping that were in resolution to this transcripting total loss having a significantly strong negative correlation with both levels of serum Anti-TPO autoantibodies (r=-0.484) as well as elevated systemic TSH levels (r=-0.426). These new findings provide insights for understanding the systemic impact of autoimmune thyroid disease/thyroid autoimmunity and that the detection or observation of transcription factor dynamics in peripheral bloodstream may offer an alternative means of assessment for the degree of inflammatory activity within the thyroid gland [12–14].

An important methodological question was raised regarding whether the PAX8 transcription factor (which has traditionally been considered to be specific to the thyroid) is biologically valid when analyzed in whole blood rather than as part of direct tissue samples [15, 16]. While direct tissue samples are still considered the "gold standard" for evaluating PAX8, some molecular diagnostic advancements (e.g., the ability to isolate and quantify cell-free mRNAs, exosomes derived from tissue, or a small fraction of circulating thyroid progenitor cells) now support the potential for analyzing those transcripts from

peripheral blood sample (PAX8) will provide valid biological information [16]. In chronic autoimmune inflammatory conditions (e.g., Hashimoto's thyroiditis), the extensive destruction of follicles by lymphocytes and complement-mediated cytotoxicity causes large amounts of cellular debris and thyroid tissue-associated transcripts to be shed into the bloodstream from the remnant of the thyroid gland [17]. Finally, numerous recent multi-omic studies have suggested that the transcriptome of systemic immune cells undergoes significant changes after receiving continuous exposure to disrupted hormone levels and provide evidence of strong biological signaling between leukocytes and damaged tissues [14, 18].

The highly significant negative correlation established between PAX8 expression and serum Anti-TPO autoantibodies ($r=-0.484$, $p<0.001$) provides strong clinical evidence for this transcriptomic-immunological coupling. PAX8 functions normally by directly binding to the promoter regions of thyroglobulin (TG), thyroid peroxidase (TPO) and the thyroid stimulating hormone receptor (TSHR) to enable full functional hormonal synthesis [15, 16, 19]. Therefore, the high level of downregulation exhibited by PAX8 has a highly damaging physiological effect. Experimental models of the Th1-driven and cytotoxic environment of autoinflammation demonstrate activation of inflammatory signaling pathways via cytokines (IFN-gamma and TNF-alpha). Such activation of the STAT/NF-kappaB signaling pathway epigenetically silences or physically inhibits PAX8 from binding to its target genes. Our data provides evidence of a model whereby antibody-mediated cytotoxicity worsens follicular cell death, which is then mathematically recorded as an amount of peripheral PAX8 mRNA reduce by an equal percentage [17, 21].

It is critical to emphasize that associations noted through cross-sectional case-control designs represent strong correlations in both statistical and biological contexts – not direct temporal causality. Since a case-control design provides only a snapshot of information (i.e., data collected over a short period) there is no way to ascertain definitively whether downregulation of peripheral PAX8 acts as either an initial driver of pathology or is a downstream consequence of continuous destruction of the thyroid gland. However, the results of multivariable binary logistic regression performed demonstrate the clinical implications of using PAX8 transcript suppression; while Anti-TPO accounts for the largest independent variance explained by classification of having the disease (OR=1.170, p=0.034), PAX8 transcript suppression is also the most sensitive biomarker available and has a 1.875 odds ratio associated with it.

Our results further build on the evidence from previous regional and international cohorts that studied chronic autoimmune inflammation [2, 3] and support the hypothesis that a progressive decrease in thyroid-specific transcriptional activity is a systemic characteristic of chronic autoimmune hypothyroidism.

■ CONCLUSION

Euthyroid controls. There was a twenty-fold difference in the fold change of the PAX8 mRNA expression level between the cases of primary autoimmune hypothyroidism (PAX8 FC=0.05) and euthyroid controls (PAX8 FC=1.00), which indicates a dramatic reduction in PAX8 expression corresponding to thyroid-specific transcriptional activity. The negative correlation with Anti-TPO and TSH levels suggest that rather than being an inherent process, transcriptional suppression was caused primarily due to immune-mediated damage to the thyroid follicular cells. A key characteristic of the autoimmune disease

process is the continued suppression of PAX8 expression, independent of sex. Supported by odds ratio calculations, patients with elevated anti-thyroid peroxidase (anti-TPO) have greater than 47 times the chance of having hypothyroidism than patients without anti-TPO antibodies; this is substantiated by excellent overall diagnostic accuracy (area under the curve (AUC) = 0.947) demonstrated via receiver operating characteristic curve analyses.

REFERENCES

1. Li J, Zhang Q. Intrathyroidal feedforward and feedback network regulating thyroid hormone synthesis and secretion. *Front Endocrinol*. 2022;13:992883.
2. Bogusławska J, Godlewska M, Gajda E, Piekłeko-Witkowska A. Cellular and molecular basis of thyroid autoimmunity. *Eur Thyroid J*. 2022;11(1):e210024.
3. Radhi SM, Al-Jumaili EF, Al-hilal MA. Evolution of thyroid autoantibodies and thyroid parameters in Iraqi hypothyroidism patients. *Iraqi J Biotechnol*. 2022;21(2):172–177.
4. Li J, Huang Q, Sun S, et al. Thyroid antibodies in Hashimoto's thyroiditis patients are positively associated with inflammation and multiple symptoms. *Sci Rep*. 2024;14:27902.
5. Batjargal K, Tajima T, Fujita-Jimbo E, et al. Functional analysis of PAX8 variants identified in patients with congenital hypothyroidism in situ. *Clin Pediatr Endocrinol*. 2022;31(4):234–242.
6. EL-Ella SSA, Khattab ESAEH, Beddah RKA, Barseem NF. Genetic variability of the paired box transcription factor PAX8 gene: guidance towards treatment strategies in a cohort of congenital hypothyroidism. *Horm Metab Res*. 2021;53(5):311–318.
7. Li M, Wang F, Wang X, et al. Genetic testing of PAX8 mutations associated with thyroid dysgenesis in Chinese congenital hypothyroidism patients. *Endokrynol Pol*. 2020;71(2):153–160.
8. Zhang QY, Ye X, Zhou Z, et al. Lymphocyte infiltration and thyrocyte destruction are driven by stromal and immune cell components in Hashimoto's thyroiditis. *Nat Commun*. 2022;13:775.
9. Kostoglou-Athanassiou I. *Hypothyroidism – New Aspects of an Old Disease*. London: IntechOpen; 2021.
10. Okab HF, Salih MB, Jarulla BA. Evaluation of CXCL 10 and IL-10 in COVID-19 pneumonia. *Pneumologia*. 2024;71(4):175–180.
11. Okab HF, Yassir HK, Majed MM. Estimation of Immunological and Physiological Parameters in Diabetic Patients with COVID-19. *Bionatura*. 2023;8(2):8–11.
12. Akyön Y, Aydemir E, Ayaz F. Hashimoto's thyroiditis as an autoimmune disorder. *Discov Med*. 2025;2(1):10–18.
13. Bogusławska J, Godlewska M, Gajda E, Piekłeko-Witkowska A. Cellular and molecular basis of thyroid autoimmunity. *Eur Thyroid J*. 2022;11(1):e210024.
14. Marotta V, Russo M, Prevete N, et al. Extracellular Vesicles and Cell-Free RNA as Biomarkers in Thyroid Diseases. *Int J Mol Sci*. 2023;24(12):10115.
15. Subhi Mahmood A, Khudair HK. Estimation of serum level and rs17855750 A/C genetic polymorphism of interleukin-27 in Hashimoto thyroiditis patients. *Iraqi J Biotechnol*. 2026;25(1):204–214.
16. Stojić M. Thyroid function disorders. *Arh Farm*. 2022;72(5):429–449.
17. Martínez-Hernández R, Blanca NS de la, Sacristán-Gómez P, et al. Unraveling the molecular architecture of autoimmune thyroid diseases at spatial resolution. *Nat Commun*. 2024;15:5895.
18. Li J, Huang Q, Sun S, et al. Thyroid antibodies in Hashimoto's thyroiditis patients are positively associated with inflammation and multiple symptoms. *Sci Rep*. 2024;14:27902.
19. Di Jeso B, Arvan P. The role of thyroid-specific transcription factors in thyroid physiology and disease. *Front Endocrinol*. 2022;13:992883.
20. Zheng S, Liu L, Liang K, et al. Multi-omics insight into the metabolic and cellular characteristics in the pathogenesis of hypothyroidism. *Commun Biol*. 2024;7:990.
21. Ibraheem RM, Hamdi RA, Kadam SM. Measurement of serum stromelysin-2 level in Iraqi patients with subclinical and clinical hypothyroidism. *J Fac Med Baghdad*. 2024;66(1):45–52.

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Prevalence, Molecular Characterization, and Antimicrobial Resistance Profiles of Multidrug-Resistant Salmonella Typhimurium Isolated from Domestic Pigeons (Columba livia)

Conflict of interest: nothing to declare.
Authors' contribution: Safaa Abd Jabah – conceptualization, data curation, investigations, resources, supervision, visualization, writing – original draft and writing – review & editing; Basim Abdalhussein Jarullah – conceptualization, data curation, project administration, resources, software, supervision, validation, and writing – review & editing.
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Abstract

Introduction. Salmonella enterica serotype Typhimurium is a zoonotic bacterium that causes foodborne disease outbreaks across the globe. Domestic pigeons (Columba livia) could be potential reservoir hosts and can be involved in the transmission of these antibiotic-resistant bacteria into the environment.
Purpose. The objective of this study was to estimate the prevalence and antimicrobial susceptibility patterns of Salmonella Typhimurium isolates from domestic pigeons in Thi-Qar Governorate, Iraq.
Materials and methods. Cloacal swabs samples from 100 domestic pigeons were collected from three distinct districts; Nasiriyah (n=60), Al-Gharraf (n=25), and Al-Shatra (n=15) during the period between October 2025 to February 2026. The isolates were characterized by using conventional bacteriology methods, VITEK 2 Compact system for identification, PCR analysis for invA, stm, tetA, and blaCTX-M genes, and antibiotic sensitivity test according to CLSI.
Results. Five isolates (5.0%; 95% confidence interval: 1.6–11.3%) were identified to be Salmonella Typhimurium. All isolates showed presence of invA and STM virulence genes. Presence of tetracycline resistance gene, tetA, and extended spectrum beta lactamase gene, blaCTX-M, was seen in all the five isolates through molecular analysis. Multidrug resistance (MDR) was seen in all isolates, while three isolates (60%) met the criteria of XDR. Complete resistance was seen against the following drugs: ampicillin, cefotaxime, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, imipenem, and ofloxacin. 80% resistance was seen against level.
Conclusion. Though the frequency of Salmonella Typhimurium infection among pigeons kept at homes in the province of Thi-Qar was low (5%), the isolation of MDR and XDR strains, containing both virulence and resistance genes, is alarming.
Keywords: salmonella typhimurium, Columba livia, antimicrobial resistance, multidrug resistance, PCR

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■ INTRODUCTION

Despite various efforts made to fight against infectious diseases around the world, Salmonella enterica continues to be one of the most prevalent zoonotic bacteria that impose serious health and economic impacts [1, 2]. Among many different serovars of this pathogen, Salmonella enterica serovar Typhimurium is quite commonly linked to food poisoning cases in both humans and animals [3]. Development of resistance of Salmonella bacteria to antimicrobial therapy has become a global issue nowadays [4]. Domesticated pigeons (Columba livia) are found across many areas both in cities and countryside and usually occur in the proximity of humans as well as other domesticated animals. The foraging habits of these birds and their ability to travel help in spreading various pathogens through contamination of food, water supply, and environment around [5, 6]. Various researches show that pigeons may act as carriers of Salmonella spp. and help spread various bacteria [7].

One of the most precise methods to confirm Salmonella isolation and distinguish clinically significant serotypes is polymerase chain reaction (PCR) [8]. The invA gene is one of the most conservative genes in Salmonella enterica serovars, and it is known to be a very reliable molecular marker of Salmonella as it plays an important role in the process of infection of intestinal epithelial cells [9]. Stm gene is used for identification of S. Typhimurium and can be applied for precise molecular identification of this serotype [10].

There has been a tremendous increase in the number of antimicrobial resistances within Salmonella bacteria in the last few decades due to the excessive use of antibiotics in both veterinary and human medicine [11]. Some of the common genes involved in causing resistance include tetA gene that causes resistance to tetracyclines by efflux action and the group of blaCTX-M genes that cause ESBL production which can hydrolyze third-generation cephalosporins [12, 13].

■ MATERIALS AND METHODS

Ethical Approval

This study was considered and granted ethical approval from the Scientific and Ethical Committee of Al-Sharqah College, Thi-Qar, Iraq (Approval No. AC-12-04-2026) on 12th April 2026.

Study design, area and period

A cross-sectional study was conducted between October 2025 and February 2026. Sampling was performed in three geographical locations: Nasiriyah, Al-Gharraf, and Al-Shatra.

Birds and sample collection

A total of 100 apparently healthy domestic pigeons (Columba livia) of both sexes were randomly sampled from households maintaining pigeons under traditional breeding systems. Birds had no documented history of antimicrobial treatment in the two weeks preceding sampling.

Cloacal swab samples were collected aseptically using sterile cotton swabs, immediately transferred to sterile transport tubes, maintained in cooled containers, and transported to the laboratory within 6 hours.

Isolation and identification of salmonella

The samples were grown in a Selenite F broth medium, incubated under aerobic conditions at 37 °C for 24 hours. The grown sample was streaked on a Xylose Lysine Deoxycholate (XLD) medium and incubated at 37 °C for 24 hours. Identification of Salmonella bacteria was done through morphological observations (white colonies with black centers), Gram staining (Gram negative bacteria) and biochemical tests [14].

Automated Identification

Presumptive isolates were confirmed using the VITEK® 2 Compact automated identification system (bioMérieux, France) according to the manufacturer's recommendations.

DNA Extraction

DNA isolation procedure involved the use of Microgen Genomic DNA Extraction Kit (Microgen, Korea) in accordance with the manufacture's protocol. Isolated DNA samples were then kept at –20 °C for molecular study.

PCR Amplification

PCR amplifications were carried out in order to confirm the presence of resistance genes. The interest genes include invA (284 bp), stm (523 bp), tetA (576 bp), and blaCTX-M (593 bp).

Table 1
Primer sequences used

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
invA	GTGAAATTATCGCCACGTTCGGGC	TCATCGCACCGTCAAAGGAACC
stm	GGAATCAATCGCCGCCAAAT	CGGTGGTGTTGATATCGCGCTGTC
tetA	GGTTCACTCGAACGACGTCA	CTGTCCGACAAGTTGCATGA
blaCTX-M	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG

PCR amplification was performed in a 25 µL reaction volume containing 12.5 µL 2× PCR Master Mix, 1 µL each of forward and reverse primers, 3 µL template DNA, and 7.5 µL

Agarose gel electrophoresis

Ethidium bromide was placed in 1.5% agarose of the PCR products, and voltage was set to 80V for 40 minutes.

Antibiotic susceptibility test

The antibiotic test was done by following the CLSI rule [15]s, using Mueller Hinton agar, and the Kirby Bauer disc diffusion style method maybe. The procedure was sort of like standard testing, you place the discs on the agar and observe inhibition zones. The antimicrobials tested included ampicillin (10 µg), amoxicillin + clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), imipenem (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ofloxacin (5 µg), trimethoprim-sulfamethoxazole (25 µg), and doxycycline (30 µg).

Data analysis

Descriptive analysis with frequencies and percentages was used for data analysis. In view of the few numbers [16].

■ RESULTS

Prevalence of Salmonella Typhimurium

Of the 100 cloacal swab samples examined, five (5.0%; 95% CI: 1.6–11.3%) were confirmed as Salmonella Typhimurium. The geographical distribution of positive samples is shown in Table 2.

Table 2
Geographical distribution of examined samples and positive isolates

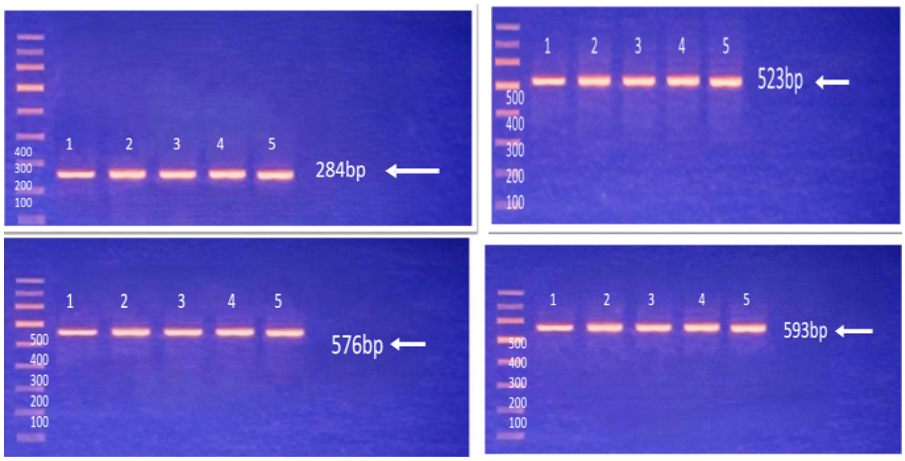
Region	Samples examined (n)	Positive isolates (n)	Prevalence (%)
Nasiriyah	60	3	5.0
Al-Gharraf	25	1	4.0
Al-Shatra	15	1	6.7

Isolation and identification

Suspected Salmonella isolates produced characteristic pale colonies with black centers on XLD agar due to hydrogen sulfide production. Microscopic exam showed Gram-negative bacilli, and honestly it looked pretty clear to us. Using the VITEK 2 Compact system, the organism was identified as Salmonella spp., with a really high level of certainty (more than 99%).

Molecular detection

Afterwards PCR amplification found invA and stm genes in all five isolates, so that pretty much confirmed the identification as Salmonella Typhimurium.The amplified fragments were of expected molecular sizes; 284 bp for invA and 523 bp for stm genes.



Agarose gel electrophoresis of PCR products for invA (284 bp), stm (523 bp), tetA (576 bp), and blaCTX-M (593 bp) in Salmonella Typhimurium isolates. Lane M: DNA ladder (100 bp); lanes 1–5: positive isolates; lane 6: negative control

All five isolates were also positive for the tetA and blaCTX-M resistance genes, with amplicons of 576 bp and 593 bp, respectively.

Antimicrobial Resistance Patterns

Antimicrobial susceptibility testing revealed high levels of resistance among the five isolates. Full resistance (100%) was found across eight antimicrobial agents. The resistance trends are summarized in Table 3.

Table 3
Antimicrobial resistance profile of Salmonella Typhimurium isolates (n=5)

Antimicrobial Agent	Susceptible n (%)	Resistant n (%)
Levofloxacin	1 (20)	4 (80)
Fosfomycin	0	5 (100)
Ampicillin	0	5 (100)
Cefotaxime	0	5 (100)
Sulfonamide	1 (20)	4 (80)
Gentamicin	0	5 (100)
Trimethoprim-sulfamethoxazole	0	5 (100)
Ciprofloxacin	0	5 (100)
Doxycycline	2 (40)	3 (60)
Amoxicillin-clavulanic acid	0	5 (100)
Imipenem	0	5 (100)
Ceftazidime	1 (20)	4 (80)

MDR and XDR classification

All five isolates (100%) were categorized as multidrug resistant (MDR), since they showed resistance to three or more antimicrobial classes. Out of those, three isolates (60%) met the extensively drug-resistant (XDR) conditions, with resistance to five, or more antimicrobial categories.

■ DISCUSSION

This present study, managed to isolate and characterize Salmonella Typhimurium from domestic pigeons in Thi-Qar Governorate, southern Iraq. Overall, the prevalence was 5.0% (95% CI: 1.6–11.3%) and it fits fairly well with earlier findings from pigeon communities in other places. Borrelli et al. [6] described a prevalence close to 4.8% in domestic pigeons from Italy, and Abd El-Tawab et al. [7] reported about 6.2% in Egypt, so there is this kind of similar trend across areas even if the settings are different. It can mean that pigeons may function as a steady reservoir for Salmonella, no matter where they are located.

Also, the isolation approach using Selenite F enrichment then XLD agar, matches what most routine microbiological protocols recommend [14]. The colony look, with pale colonies plus dark or black centers, points toward hydrogen sulfide production. That trait is often treated as a dependable phenotypic clue when you want to identify Salmonella. After that, the VITEK 2 Compact system gave a quick confirmation and it was in line with prior work showing that automated platforms can be very useful for identifying Enterobacteriaceae [17]. Molecular detection confirmed the presence of the invA gene in all isolates, which is often taken as a genus specific marker for

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Salmonella enterica [9]. Amplification of the stm gene in all five isolates, confirmed their identity as Salmonella Typhimurium, and this fits with earlier work that used this serovar specific marker [10].

All isolates also carried the tetA and blaCTX-M resistance genes. The tetA gene, encodes an efflux pump that provides resistance to tetracycline as well as related compounds [18]. As for blaCTX-M, it is among the most broadly distributed ESBL genes in Enterobacteriaceae, and it is repeatedly linked with plasmid mediated horizontal gene transfer [12, 13]. The antimicrobial susceptibility results showed, in a frankly worrying way, very high levels of resistance. The full resistance (100%) we saw to ampicillin, cefotaxime, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, imipenem, and ofloxacin suggests that the recovered isolates are resistant to a broad range of first line, and also critically important, antimicrobials. The complete resistance to ciprofloxacin is especially troubling since fluoroquinolones are widely regarded as critically important when it comes to managing severe salmonellosis [19].

In the current study, all isolates met the requirements for MDR, and 60% also met the requirements for XDR. These observations match the wider, worldwide pattern of increasing antimicrobial resistance in Salmonella isolates [4, 11]. Still, the share of XDR isolates (60%) in our work is higher than what's usually described in other investigations. For example, one recent systematic review found XDR Salmonella prevalence, roughly, from 5% to 25% across regions [20].

Another point that really stands out is the co-existence of virulence genes (invA, stm) alongside resistance genes (tetA, blaCTX-M) within the same isolates. This pair, or rather this overall mix, implies these isolates may be able to trigger disease while also resisting several antimicrobial agents at once. Similar results have been reported elsewhere too, including work from other countries where Salmonella from avian sources carried both kinds of markers, virulence determinants plus resistance genes [8, 21].

■ CONCLUSION

This present study kind of confirms that domestic pigeons from Thi-Qar Governorate, southern Iraq, have Salmonella Typhimurium, they also seem to carry both types of genetic bits, the virulence-associated genes (invA, stm) plus antimicrobial resistance genes (tetA, blaCTX-M). Even though the detected prevalence looks low, about 5%, finding multidrug-resistant isolates at 100% and extensively drug-resistant ones at 60% is still worrying for public health, you know. The resistance levels that were observed were high especially against critically important antimicrobial agents, like fluoroquinolones, third-generation cephalosporins, and carbapenems. That pattern points toward pigeons acting like reservoirs for resistant Salmonella strains, sort of a harbor type situation. And when the virulence genes together with resistance genes turn up in the very same isolates, then the possible hazard for human health and animal health becomes even more significant, honestly it gets more serious than before.

REFERENCES

1. Ferrari RG, Rosario DK, Cunha-Neto A, et al. Worldwide Epidemiology of Salmonella Serovars in Animal-Based Foods: a Meta-analysis. *Appl Environ Microbiol.* 2019;85(14):e00591–19.
2. World Health Organization. Global antimicrobial resistance surveillance report 2024. Geneva: WHO; 2024.
3. European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union One Health 2023 zoonoses report. *EFSA J.* 2024;22(2):e8554.
4. Murray CJ, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2021. *Lancet.* 2024;404(10469):2041–2053.
5. Silva N, Igrejas G, Rodrigues P, et al. Antimicrobial resistance in wild birds. *Antibiotics.* 2020;9(6):324.
6. Borrelli L, Dipineto L, Pace A, et al. Prevalence and antimicrobial resistance of Salmonella spp. isolated from domestic pigeons in Italy. *Vet World.* 2021;14(5):1234–1240.
7. Abd El-Tawab AA, El-Hofy FI, El-Bauomy AA. Prevalence of Salmonella in pigeons and its public health importance. *Benha Vet Med J.* 2023;44(1):45–52.
8. Yang Y, Zhang X, Li J. Molecular detection of virulence genes in Salmonella isolates. *Food Control.* 2022;132:108567.
9. Rahn K, De Grandis SA, Clarke RC, et al. PCR detection of the invA gene in Salmonella. *Mol Cell Probes.* 1992;6(4):271–279.
10. Shanmugasundaram M, Radhika M, Murali HS, Batra HV. Detection of stm gene in Salmonella Typhimurium. *Foodborne Pathog Dis.* 2009;6(10):1275–1281.
11. Larsson DGJ, Flach CF, Laxminarayan R. Antimicrobial resistance in the environment. *Lancet Planet Health.* 2024;8(1):e45–e55.
12. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases. *J Antimicrob Chemother.* 2017;72(8):2145–2155.
13. Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: An update. JAC-Antimicrob Resist. 2024;6(1):dlae012.
14. Quinn PJ, Markey BK, Leonard FC, et al. Veterinary microbiology. 3rd ed. Chichester: Wiley-Blackwell; 2016.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 35th ed. CLSI supplement M100. Wayne: CLSI; 2025.
16. Ameen IA, Okab HF. Phyto-activity of Syzygium aromaticum extract against pathogenic bacteria isolated from chronic tonsilitis patients. *Rom J Infect Dis.* 2024;27(1):5–10.
17. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: A review. *Clin Infect Dis.* 2009;49(11):1749–1755.
18. Randall LP, Cooles SW, Osborn MK, et al. Tetracycline resistance genes in bacteria. *J Antimicrob Chemother.* 2004;53(2):208–216.
19. Hooper DC, Jacoby GA. Mechanisms of fluoroquinolone resistance. *Microbiol Spectr.* 2024;12(2):e02345–23.
20. Wang X, Li Y, Zhao S. Prevalence of XDR Salmonella in Asia: A systematic review. *J Global Antimicrob Resist.* 2024;35:45–56.
21. Elkenany R, Abdelrahim A, El-Geddawy A. Molecular characterization of Salmonella serovars from chickens. *J Appl Microbiol.* 2023;134(2):lxac045.