https://doi.org/10.18502/jad.v20i1.21964

Original Article

Association of Head Lice Infestation with Staphylococcal Dysbiosis: Molecular Identification of *Pediculus capitis* and Staphylococcal Profiling in School Children

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(Received 26 Jan 2026; accepted 09 Mar 2026)

Abstract

Background: Head lice infestations are a widespread health problem among school-aged children globally. Nevertheless, the importance of lice as initiators of scalp microbiome changes and as causes of secondary bacterial superinfections remains poorly understood. The paper aims to examine the PCR-based identification of head lice and to assess the epidemiological relationship between head lice infestation and scalp colonization by *Staphylococcus* species.

Methods: An analytical cross-sectional study was conducted on 100 primary school children (50 infested and 50 controls) aged between 5 and 12 years in the governorate of Nineveh (Iraq). The molecular identification of head lice was performed by amplifying the COX1 gene, and the comprehensive Staphylococcal profiling of scalp swabs was performed using culture and 16S rRNA gene amplification.

Results: Molecular analysis using COX1 gene specific amplification showed the presence of *P. humanus capitis* in 93.9% of the collected samples. The microbiological tests showed profound staphylococcal dysbiosis: *Staphylococcus aureus* was detected in 74% of infested children and absent in the control group (0%), indicating a highly significant association (χ^2=58.73, p<0.001). Conversely, the commensal *Staphylococcus epidermidis* was found predominantly in healthy controls (66%) but significantly less frequently in infested children (26%).

Conclusion: The pathogenic *S. aureus* prevails on the scalp of children with head lice with a striking shift, which illustrates a clinically significant interaction of ectoparasitic infestation with staphylococcal dysbiosis. The results also suggest that pediculosis is a risk factor for *S. aureus* overgrowth and emphasize the need for combined treatment strategies that address lice and bacterial complications.

Keywords: Pediculosis capitis; Scalp microbiome; Staphylococcal dysbiosis; COX1 gene; 16S rRNA gene

Introduction

Head lice (*Pediculus capitis*) are obligate human ectoparasites that feed on the scalp blood multiple times daily (1) and exhibit high prevalence rates, particularly near close contact with children (2). Moreover, about 6–12 million people (including children) are infested with head lice in the United States each year, corresponding to 10–40% prevalence among school-aged children (3). Consequently, pediculosis capitis is a public health concern worldwide, particularly among school-aged children (4).

The primary clinical presentation is severe scalp pruritus, resulting from hypersensitivity to injected louse saliva during feeding (5). Furthermore, the condition causes significant scalp irritation, pruritus, sleep disturbance and social stigma, negatively influencing children's well-being and academic performance (6). However, socioeconomic conditions, overcrowding and hygiene habits are likely to affect the prevalence (2).

In addition to its parasitic effect, there has

https://doi.org/10.18502/jad.v20i1.21964

been growing interest in the possibility of *Pediculus capitis* to be a host and potentially a carrier of pathogenic bacteria. Accordingly, several studies have identified *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acinetobacter* spp. and other opportunistic bacteria as associated with lice, suggesting potential interactions among bacteria, lice and the host (7, 8).

Scratching itchy skin is common and can disrupt the epidermal barrier. Consequently, this breach may create an opportunity for secondary bacterial pathogens, which can lead to complications, including impetigo, pyoderma and in extreme cases, systemic infection (9). The scalp microbiome is diverse, although the genus of *Staphylococcus* is some of the most common colonizers. Notably, the common commensal is *Staphylococcus epidermidis*, but *Staphylococcus aureus*, which is also a frequent nasal and skin colonizer, is a major opportunistic pathogen (10). *Staphylococcus aureus* is a coagulase-positive bacterium that causes skin infections, abscesses and more serious diseases, but *S. epidermidis* is a coagulase-negative *Staphylococcus* that is commonly associated with wound infections and colonization of prosthetic devices (11). These bacteria may be acquired by lice when they feed on the scalp, and it has been found that the presence of staphylococci on lice can lead to secondary bacterial infections in infested persons (5). As a matter of fact, *S. aureus* and group A streptococci have been observed to be harbored on the surfaces of lice and then be transmitted to human beings via bites or scratched skin (mechanical transmission) (12).

For this study, we define dysbiosis narrowly as a measurable shift in dominant *Staphylococcus* species-specifically, the displacement of commensal *S. epidermidis* by pathogenic *S. aureus*-rather than a comprehensive alteration of the entire scalp microbiome. Nevertheless, the traditional methods of lice identification are largely based on morphological evaluation, which may be inaccurate for identifying closely related lice species or for detecting genetic variation. Molecular identification methods, especially amplification of the mitochondrial COI gene, provide powerful, sensitive and specific means of accurate identification of lice, classification, taxonomic validation and population genetics analyses, thereby overcoming the shortcomings of morphological identification (13). For example, species-specific genetic markers can be used to distinguish head from body lice via PCR assays. Similarly, 16S rRNA gene amplification is also an efficient technique for confirming bacterial species (14).

Therefore, this research was conducted to validate the PCR-based identification of *Pediculus capitis* in school children, as well as to determine the prevalence and diversity of related *Staphylococcus* species, thereby clarifying the possibility of co-transmission of bacteria in schools.

Materials and Methods

Study design and setting

This analytical cross-sectional study was conducted over three months (April–July 2025) in primary schools across different districts of Nineveh governorate (Iraq). Informed consent was obtained from legal guardians and verbal assent was obtained from participating children. A total of 100 children were recruited via random sampling from 17 primary schools (urban and rural): 50 infested (aged 6–10 years) and 50 controls (aged 5–12 years) without pediculosis. Infestation status was determined via visual inspection and combing after consent was secured.

Study population and sample collection

Lice collection: Fifty live adult lice were collected from the 50 infested children using fine-toothed combs and sterile forceps. Lice were immediately preserved in 70% ethanol in Eppendorf tubes for subsequent DNA extraction and identification.

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Published Online: March 31, 2026

https://doi.org/10.18502/jad.v20i1.21964

Scalp swabs: Scalp swabs were collected from all 100 children (50 infested, 50 control). Crucially, as infestations were observed exclusively among female students during screening, the study population was limited to female participants in both groups to maintain comparability and minimize gender-related confounding. Swabs of the occipital, retroauricular and frontal scalp areas (preferred lice habitats) were immediately placed in Amies transport medium and transported to the laboratory within 4 hours. Concurrently, a structured questionnaire gathered demographic data (age, sex, hair length, infestation history, residence and household size) and clinical examination was used to record infestation severity (mild: <10 lice; moderate: 10–50 lice; severe: >50 lice).

Inclusion and exclusion criteria

Children aged 5–12 years in public primary schools were eligible. The patient group comprised children with clinically confirmed head lice infestation; the control group comprised children from the same schools with no clinical evidence or history of pediculosis at examination. All participants were required not to have received pediculicidal treatment within the preceding four weeks. **Exclusion criteria included:** Use of systemic/topical antibiotics, antibacterial/antifungal agents, antiseptic scalp preparations, or medicated shampoos (including anti-dandruff/antimicrobial) within the previous four weeks; active scalp dermatological conditions (e.g., impetigo, eczema, psoriasis); chronic systemic diseases; refusal of consent/assent; or lice specimens unsuitable for molecular analysis.

Morphological identification

Primary morphological identification of collected lice was based on key external features: body segmentation, three pairs of legs with terminal claws, absence of wings and general body size.

Molecular identification of *Pediculus capitis*

DNA extraction and molecular identification of lice

Genomic DNA was extracted from whole head lice using a commercial silica column-based kit (Genesand Biotech, China) following the manufacturer's instructions with minor modifications. Each louse was washed three times with sterile phosphate-buffered saline (PBS) to remove external contaminants. Briefly, individual lice were homogenized in lysis buffer with sterile pestles and digested with proteinase K. Extracted DNA was eluted in TE buffer and stored at -20 °C. DNA concentration and purity were assessed using spectrophotometry (NanoDrop). Due to resource limitations, molecular analysis was performed on a randomly selected, representative subset of 33 of the 50 collected lice specimens. Random selection was conducted using a simple random sampling approach to ensure that each specimen had an equal probability of inclusion. The selected subset was considered representative of the collected samples because all lice originated from the same study population and were morphologically consistent with *Pediculus capitis*.

PCR amplification

Molecular detection of *Pediculus capitis* targeted a partial fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene (COX1 gene) using universal primers LepF: (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR: (5'-TAAACTTCTGGATGTCC AAAAAATCA-3') (15). PCR reactions were performed in 25 µL volumes containing 12.5 µL Mastermix, 5 µL DNA template, 2 µL primers and 5.5 µL deionized distilled water. Thermal cycling conditions were: initial denaturation at 95 °C for 5 minutes; 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 52 °C for 40 seconds and extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 10 minutes. PCR products (expected size ~658 bp) were visualized on 1.5% agarose gels stained with safe green under UV transillumination.

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Isolation and identification of *Staphylococcus* species

Bacterial isolation was performed on blood agar (BA) and incubated aerobically at 37 °C for 24 to 48 hours. Preliminary identification utilized standard methods: Gram staining (Gram-positive cocci in clusters), catalase test (positive), coagulase test (to distinguish *S. aureus* from CoNS) and oxidase test (negative). Isolates were further subcultured on Mannitol Salt Agar (MSA); yellow colonies indicated mannitol fermenters, while pink indicated non-fermenters. Stock cultures were maintained in 20% glycerol at −20 °C (16).

Molecular identification

Bacterial genomic DNA was extracted using a commercial kit (Foregene, China) per instructions. The 16S rRNA gene was amplified using universal bacterial primers 342F (5'-CTACGGGGGGCAGCAG-3') and 806R (5'-GGACTACCGGGGTATCT-3') (17). PCR conditions included: initial denaturation at 94 °C for 5 minutes; 35 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 1 minute; followed by final extension at 72 °C for 10 minutes. The expected amplicon size was ~465 bp.

PCR quality control

Appropriate positive and negative controls were included for each PCR run. The positive control for COX1 amplification was previously confirmed head lice DNA and for 16S rRNA amplification, genomic DNA from a known *Staphylococcus* spp. isolate was used. A negative control (nuclease-free water instead of template DNA) was included in every batch to monitor a potential contamination. To minimize cross-contamination, DNA extraction, PCR setup and post-amplification analysis were performed in physically separated areas using sterile filter pipette tips and dedicated equipment.

Statistical analysis

Data were analyzed in SPSS v27. Demographic variables, infestation prevalence and bacterial species distribution were analyzed using descriptive statistics. Categorical variables were presented as frequencies and percentages, while continuous variables were reported as means ± standard deviations. Associations between two categorical variables were examined using the chi-square test or Fisher's exact test. To estimate effect size with 95% confidence intervals, a corrected odds ratio was calculated using the Haldane-Anscombe correction (addition of 0.5 to each cell). Statistical significance was set at $p \leq 0.05$.

Results

A total of 100 children were included in the study, with 50 clinically infested and 50 non-infested controls. Notably, all participants in the current study were female, as head lice were detected only in girls during the screening period. Regarding age, patients were 6-10 years old (7.44±1.31 years), whereas controls were 5–12 years old (6.96±2.01 years). The age distributions were comparable between groups (Patient: 7.44±1.31 years, range 6−10; Control: 6.96±2.01 years, range 5−12); while the mean ages were not significantly different, control variability was greater.

The bacteriological culture findings showed clear differences between the patient and control groups. *Staphylococcus aureus* was identified in 37 (74%) of the 50 children in the patient group, making it the most prevalent isolate (Figs. 1 and 2). The remaining 13 (26 %) patient samples were identified as *S. epidermidis*. In contrast, the control group showed a reversal: *S. epidermidis* was found in 33 (66 %) of 50 children. Critically, 17 control samples showed no bacterial growth and *S. aureus* was not detected in any control subject (Table 1).

Hair characteristics and infestation severity varied between groups (Table 2). Most subjects had medium hair (33; 66%), followed by long (11; 22%) and short (6; 12%). The predominant hair color was black (31; 62%), fol-

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Published Online: March 31, 2026

https://doi.org/10.18502/jad.v20i1.21964

lowed by brown (15; 30%) and light brown (4; 8%). The majority of children resided in rural areas (39; 78%) compared to urban areas (11; 22%). In terms of severity, moderate infestation was most prevalent (32; 64%), with light infestation in the remaining 18 (36%). History of prior infestation was reported by 12 (24%) subjects. Statistical analysis showed no significant relationship between infestation intensity and hair length (p=0.128), hair color (p= 0.289), or prior infestation history (p= 0.543). However, children in rural areas were more likely to have a moderate infestation than those in urban areas (p= 0.047). Furthermore, *S. aureus* infection was significantly associated with moderate infestation compared with *S. epidermidis* colonization (p= 0.022).

These findings indicate a markedly higher prevalence of *S. aureus* among children with head lice than among non-infested controls. A chi-square test confirmed a highly significant association between *S. aureus* isolation and the patient group (χ^2= 58.73, p< 0.001). Given the absence of *S. aureus* in the control group, the corrected odds ratio (Haldane–Anscombe method) was calculated, revealing that children with head lice had 138.43 times higher odds of *S. aureus* colonization compared with controls (95% CI: 17.44–1098.97), indicating a very strong association. PCR amplification of the 16S rRNA gene was successful in all bacterial isolates (Fig. 3). Molecular analysis of the randomly selected subset (33/50 lice) showed successful amplification of the COX1 gene in 31 samples (93.9%), while 2 samples (6.1%) failed to amplify, confirming the presence of *Pediculus capitis*. Molecular analysis was conducted on a randomly selected subset of 33 out of the 50 collected lice specimens.

Table 1. Demographic characteristics and bacterial distribution among patients with pediculosis capitis and healthy controls in Mosul, Iraq, 2024–2025

Group (N)	Age (Mean±SD)	Age group	Isolated bacteria		
			S. aureus	*S. epidermidis*	No growth
Patients (50)	7.44±1.31	6–10 years	37 (74%)	13 (26%)	0 (0%)
Controls (50)	6.96±2.01	5–12 years	0 (0%)	33 (66%)	17 (34%)

Table 2. Distribution of pediculosis capitis cases according to hair characteristics, residence, infestation intensity and previous infestation history among study participants in Mosul, Iraq, 2024–2025

Variables	N (%)
Hair Length	
Short	6 (12%)
Medium	33 (66%)
Long	11 (22%)
Hair Color	
Black	31 (62%)
Brown	15 (30%)
Light Brown	4 (8%)
Residence	
Rural	39 (78%)
Urban	11 (22%)
Intensity of infestation	
Low	18 (36%)
Moderate	32 (64%)
High	0 (0%)
History of previous infestation	
No	38 (76%)
Yes	12 (24%)

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Published Online: March 31, 2026

https://doi.org/10.18502/jad.v20i1.21964

Fig. 1. *Staphylococcus* spp on blood agar

Fig. 2. *Staphylococcus aureus* on MSA

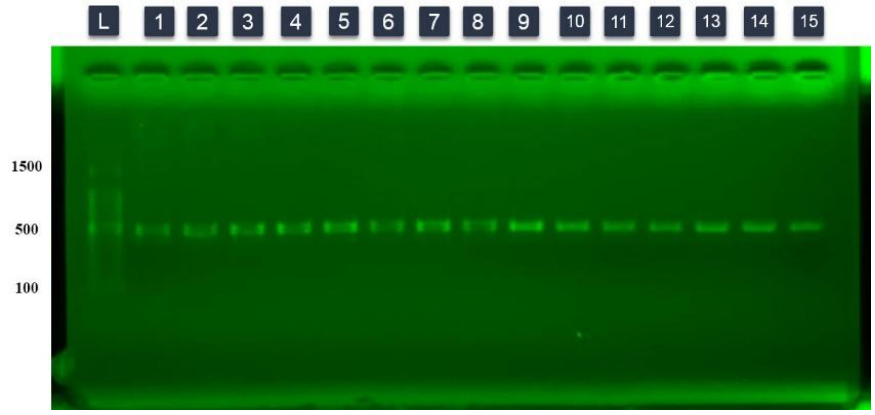

Fig. 3. The result of the 16S rRNA gene amplification for *Staphylococcus* spp. with a band size of ~465 bp. The result was obtained by electrophoresis on 1.5% agarose 5 volts/cm. L: DNA ladder; lanes 1–15: PCR products obtained from the *Staphylococcus* samples having 16S rRNA gene

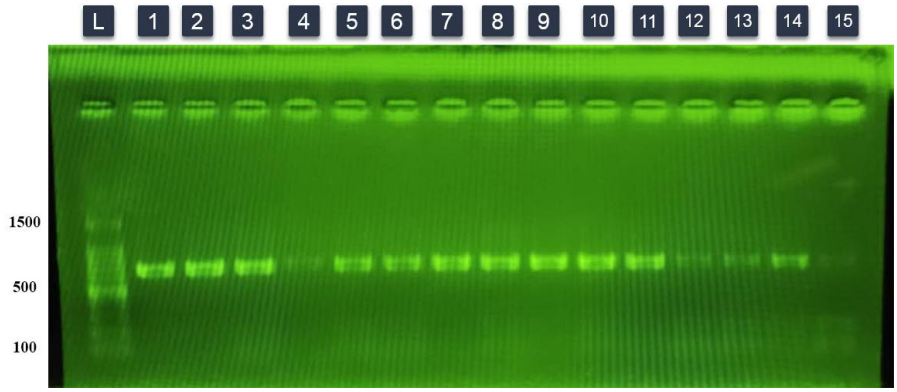

Fig. 4. The result of the cox1 gene amplification for head lice with a band size of ~658 bp. The result was obtained by electrophoresis on 1.5% agarose 5 volts/cm. L: DNA ladder; lanes 1–15: PCR products obtained from the head lice samples having cox1 gene

http://jad.tums.ac.ir
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https://doi.org/10.18502/jad.v20i1.21964

Discussion

The present study investigated the molecular identification of *Pediculus capitis* and its association with colonization by *Staphylococcus* species among primary school children. Overall, the findings reveal a significant epidemiological association between head lice infestation and scalp colonization with *Staphylococcus aureus*. In the current study, the higher prevalence in girls may reflect behavioral, social, or hair-related factors that increase exposure or facilitate transmission (18). Moreover, in Delie's systematic review and meta-analysis, girls were approximately 3.71 times as likely as boys to have head lice (2). Furthermore, the age distributions were similar in both study groups, with the patient and control populations having means of 7.44±1.31 and 6.96±2.01, respectively, thereby minimizing age-related confounding on bacterial colonization or immune response. Importantly, the age range fits with the optimal rate of prevalence of pediculosis capitis as it is prevalent in children under the ages of 11 years old, resulting from close-contact activities, which are common in school and play settings (19, 20). Head lice transmission is primarily through direct head-to-head contact, which is common among young children, especially during play, group activities and when using shared facilities (21).

The most striking finding was the significantly higher level of *Staphylococcus aureus* colonization in lice-infested children compared to non-infested controls. In particular, 37 (74 %) infested children harbored *S. aureus* on their scalps, a pathogen entirely absents in the control group (χ^2= 58.73, p<0.001). This strong statistical association suggests that head line create a favorable microenvironment for *S. aureus* colonization through several means. Initial variations could stem from group differences (behavior, hygiene, infestation intensity), but methodological factors likely played a more substantial role in the control group's zero detection. The study exclusively used scalp swabs; conversely, most literature bases carriage rates on anterior nasal swabs-the primary ecological niche for *S. aureus* (22). Furthermore, culture methods can underestimate true carriage by missing low-density or intermittent colonization. The control group's small size and strict exclusion criteria (e.g., recent antimicrobial use) further reduced the chance of detection. Therefore, the absence in controls likely reflects sampling sensitivity and selection bias rather than true non-carriage in the broader population (23). However, the pathophysiological basis of this association and dominance in infested children can be attributed to multiple interrelated factors. First, lice feeding induces micro-wounds and inflammatory reactions in scalp tissue, weakening the skin barrier and creating more space for bacterial colonization (24). In addition, saliva injected during feeding by lice contains anticoagulant and immunomodulatory substances that could further promote bacterial adherence and development (25). Second, lice infestation causes pruritus, which causes repetitive scratching that results in additional skin damage and exposes the scalp microenvironment to bacteria from the hands and subungual spaces (26). Third, lice feces, exuviae and nit cement on hair shafts and the scalp surface can alter local microbiome composition, local pH and nutrient availability, thereby providing a favorable environment for *S. aureus* relative to commensal organisms (5). However, although prior literature suggests that head lice may harbor or mechanically transmit bacterial pathogens, the present study did not assess the internal or external microbiome of lice specimens. Therefore, the findings should be interpreted as demonstrating an association between head lice infestation and increased scalp colonization with *Staphylococcus aureus*, rather than evidence of direct transmission by the parasite. The observed relationship may instead reflect infestation-induced disruption of the skin barrier,

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inflammatory changes, or scratching-related microtrauma that facilitate bacterial overgrowth (8). On the other hand, the predominant prevalence of *S. epidermidis* in the control group (33: 66%) indicates that this coagulase-negative *Staphylococcus* is part of the normal skin microbiota and is an established component of the skin microbiome, with low pathogenicity in immunocompetent persons (27). Moreover, *S. epidermidis* is a commensal organism, and its presence in the control group aligns with expected colonization patterns in healthy children and supports the validity of the sampling and culture methodology employed in this study (28). Meanwhile, the absence of bacterial growth in approximately one-third of controls may reflect either genuinely low bacterial loads below the detection threshold of culture methods or effective antimicrobial properties of the intact scalp barrier in healthy, non-infested children (29). From a molecular perspective, the universal detection of 16S rRNA genes in all bacterial isolates serves as an important positive control, confirming the viability of bacterial DNA and confirming the integrity of the molecular workflow as well as the reliability of the DNA extraction and amplification procedures.

Similarly, the collected lice specimens were identified by amplifying the COX1 gene. Of the 33 samples that underwent DNA extraction, 31 (93.9%) yielded positive PCR amplification, producing amplicons of the expected size. This success rate indicates the accuracy of morphological identification and demonstrates the effectiveness of molecular techniques in pediculosis surveillance. Furthermore, the COX1 gene is highly conserved and is generally considered a reliable molecular marker for identifying arthropods, as it exhibits sufficient sequence variation and is well represented in reference databases (30). However, the 2 (6.1%) PCR amplification failures could be explained by technical issues such as DNA degradation (especially in older/desiccated samples), insufficient louse biomass and poor DNA quality or quantity. Additionally, some samples may have been affected by PCR inhibitors generated by components of lice cuticles or environmental contamination, thereby impeding polymerase activity (31). Finally, this research has significant implications for clinical practice and public policy. First, medical professionals must maintain vigilance for bacterial superinfection, particularly *S. aureus*, by regularly examining infested children's scalps for signs like pustules or crusts (32). Second, management should integrate pediculicidal treatment with interventions to prevent bacterial complications, such as using topical antiseptics or systemic antibiotics when necessary, alongside wound-care education, hand hygiene and discouraging scratching (33). Third, these findings confirm the relevance of school-based screening and education to prevent pediculosis. The identified age group (mostly 6–10 years) is in the early years of primary school, where close physical contact during play and learning facilitates efficient disease transmission in the classroom. It could be possible to encourage and integrate head lice screening into regular school health programs, together with providing information about good hygiene and early treatment, which will reduce infestation levels and the risk of secondary bacterial complications (34).

Limitations of the study

A number of limitations should be considered when interpreting the findings of this study. First, all participants were female, as infestation was detected exclusively among girls during screening. Although this approach ensured group comparability and minimized gender-related confounding, it limits the external validity of the findings. Therefore, the results may not be generalizable to male schoolchildren and further studies including both sexes are recommended to confirm the observed associations. Second, the cross-sectional design captures colonization status at a single time point, precluding the establishment of temporal

https://doi.org/10.18502/jad.v20i1.21964

relationships before, during, or after infestation. Third, the research failed to assess potential confounding factors-such as socioeconomic status and hygiene practices-that could affect lice infestations and bacterial colonization patterns. Fourth, no molecular analysis of the lice was conducted to assess possible carriage of pathogenic bacteria, which would have provided direct evidence of vector transmission. Lastly, molecular analysis was limited to a random subset of 33 specimens due to resource constraints, which may limit the generalizability and precision of the reported COX1 amplification rate.

Conclusion

This paper demonstrates a strong association between *Pediculus capitis* infestation and colonization with *Staphylococcus aureus* in primary school children. Overall, the predominance of *S. aureus* in patients with pediculosis highlights the polymicrobial nature of the condition and its associated complication risks. Therefore, a longitudinal study is required to examine temporal relationships, treatment effectiveness and the potential for normalizing scalp flora.

Acknowledgements

We thank the school administrations, children and their legal guardians for their cooperation and participation in this study. Furthermore, our special acknowledgement goes to the General Directorate of Education in Nineveh, which permitted the conduct of this research and provided access to the primary schools.

Conflict of interest statement

The authors have declared that no competing interests exist.

Ethical consideration

The study protocol was approved by the College of Health and Medical Techniques/Baghdad (Ref. 3/2206, 10/4/2025) and the General Directorate of Education in Nineveh (Ref. 1314, 15/4/2025).

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