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Molecular characterization of mutations in methicillin resistant coagulase negative *Staphylococcus pasteuri* from various food samples

Zahraa A. Ahmed
Department of Biotechnology, College of Sciences, University of Anbar, Ramadi, Iraq. E-mail: zahraaabdahmed@uoanbar.edu.iq

Asmaa S. Ahmaed
College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq. E-mail: asmaa.s@coagri.uobghdad.edu.iq

Huda M. Mahmood
* Corresponding author. Department of Biotechnology, College of Sciences, University of Anbar, Ramadi, Iraq. E-mail: huda.mahmood@uoanbar.edu.iq

Abstract

Objective

Staphylococci are naturally present in the bodies of animals and humans, particularly in the digestive and respiratory systems. *Staphylococcus spp*. are widespread microorganisms in food environments, having been isolated from a variety of products, including meat, dairy, and ready-to-eat foods. This aim of the current study was to isolate and identify *Staphylococcus pasteuri* from various food products (meat and cheese) and food handlers, and to characterize their antibiotic resistance profiles and genetic mutations using the whole-genome data.

Materials and methods

A total of 58 food samples (fresh meat, cooked meat, and cheese) and 13 hand swabs from food handlers were collected in Baghdad, Iraq. The Kirby-Bauer disc diffusion method was used to screen isolates for methicillin resistance. Output of this method was confirmed via the VITEK 2 system. Selected resistant strains were sequenced using Illumina technology to study whole-genome sequencing (WGS) and to analyze resistance genes and single-nucleotide polymorphisms (SNPs).

Results

Staphylococcus pasteuri was successfully isolated from the tested food samples and food handlers. The results highlighted its potential transmission through the food chain. A high prevalence of multi-drug resistance traits was determined by considering phenotypic and molecular characterization. Key metabolic pathways, including NAD+ dependent enzymes and the phosphoenolpyruvate phosphotransferase system (PTS) were revealed by WGS analysis. These results indicated high metabolic versatility and adaptability to different type of food

environments. Moreover, significant plasticity was revealed by genomic analysis. These results were characterized by the presence of mobile genetic elements, resistance determinants, and numerous single-nucleotide polymorphisms (SNPs). Notably, high-impact mutations were detected within uncharacterized domains (DUFs), specifically DUF3310 and DUF771, which may possess novel regulatory functions.

Conclusion

The results of the present study showed that S. *pasteuri* has a highly flexible and comprehensive genomic architecture. This enables it to survive, adapt, and spread antibiotic resistance through the food chain. This is a significant public health concern that must be addressed.

Keywords: food safety, methicillin resistance, single-nucleotide polymorphisms (SNPs), *Staphylococcus pasteuri*, whole-genome sequencing

Paper Type: Research Paper.

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Introduction

Staphylococci are ubiquitous and resilient microorganisms. They are able to thrive in diverse and harsh environmental conditions. They are common commensals of the natural flora. They naturally colonize the skin, mucous membranes, and the digestive and respiratory tracts of humans and animals. Consequently, these bacteria are easily transmitted to food products. This transmission takes place via infected food handlers, contaminated contact surfaces, or cross-contamination during preparation and packaging processes. Due to their high tolerance to environmental stressors, staphylococci frequently contaminate the microflora of ready-to-eat foods (Chajęcka-Wierzchowska et al., 2023). Staphylococcal species are widespread across various food sectors. They are regularly isolated from meat, dairy products, ready-to-eat meals, and pastries. They represent a major source of food contamination with critical public health implications (Yaseen et al., 2020; Moges et al., 2024). Recent surveillance indicates that these foodborne contaminants possess an alarming capacity to acquire and disseminate antibiotic resistance genes, escalating global public health concerns (Jiang et al., 2024). In this context, coagulase-negative staphylococci (CoNS), such as *Staphylococcus pasteuri*, have garnered increasing attention. Previously dismissed as non-pathogenic contaminants, recent evidence

highlights CoNS as critical genetic reservoirs for resistance factors, capable of transferring these elements to highly virulent pathogens like *Staphylococcus* aureus (Nocera & De Martino, 2024). The isolation of S. *pasteuri* from diverse food matrixes, coupled with its multi-drug resistance profiles, underscores its active role in the dynamics of resistance dissemination within the food chain (Kahraman Yılmaz & Berik, 2024). Methicillin resistance represents one of the most formidable therapeutic challenges in staphylococcal infections. This phenotype is fundamentally driven by the mecA gene (or its homologues). This gene encodes a penicillin-binding protein with low affinity for beta-lactams (PBP2a). Thus, it confers resistance to nearly all beta-lactam antibiotics (Hemmati et al., 2024). Methicillin-resistant CoNS strains are frequently multi-drug resistant. They often co-harboring additional resistance determinants such as blaZ, tet, and erm. They protect the bacteria against various antibiotic classes (Jiang et al., 2024). At the genetic level, antibiotic resistance evolves through two ways. The first way takes place via spontaneous chromosomal mutations. The second method perform through horizontal gene transfer mediated by mobile genetic elements (MGEs) like plasmids and transposons. The proliferation of these resistant genotypes is further accelerated by the unrestricted use of antibiotics in both human medicine and animal husbandry (Titouche et al., 2025). Moreover, biochemical tests are incapable of distinguishing different types of microorganisms, such as bacteria, viruses, and parasites (Ahsani et al. 2010). Genomic techniques such as PCR and sequencing are the most modern practical technology in diagnosing infectious diseases and compared with classical techniques, it has been shown to be more rapid, with results obtained in a few hours, and also more reliable (Mohammadabadi et al., 2004). Genomic techniques allow a faster identification directly from clinical samples (Shahdadnejad et al. 2016). Furthermore, comparative genomics shows that foodborne *Staphylococcus* isolates frequently share genetic lineages with clinical strains. They emphasize the fluid transition and dissemination of resistance between the food industry and clinical settings (Anas et al., 2025). Therefore, the aim of this study was to evaluate the molecular characteristics and specific mutational profiles of methicillin-resistant S. *pasteuri* isolated from food to decode the mechanisms of resistance development, mitigating associated food safety risks, and establishing effective containment strategies.

Materials and Methods

Isolation and identification of bacteria: Bacterial isolation samples were collected from various food sources in several local shops and public restaurants in Baghdad. These included 20 samples of fresh local meat, 17 samples of cooked meat, and 21 samples of cheese. All samples were collected between January 2025 and July 2025, placed in clean and sterile containers, and transported to the laboratory for bacterial isolation. The isolation process, for microbiological analyses was carried out in the Graduate Studies, Biotechnology Laboratory, Food Science Department, using the standard spread-plate and pour-plate techniques as described by López Gutierrez et al., (2017).

Food handler swab collection: Thirteen hand swab samples were collected from food handlers at various local shops and restaurants in Baghdad. The hands were examined using a sterile cotton swab, applying regular pressure and rotating the swab continuously. These samples,

placed in a sterile container, were immediately transported to the laboratory in an icebox and examined within one hour (López Gutierrez et al., 2017).

Testing the resistance of bacterial isolates to the antibiotic methicillin disc: Eighty-four purified isolates underwent antibiotic susceptibility testing to determine the susceptibility of *Staphylococcus* isolates to methicillin using the Kirby-Bauer test with Mueller-Hinton agar medium, as described in (Benson, 2002; Morello et al., 2006).

Viteck confirmation: *Staphylococcus* bacteria isolates, particularly *Staphylococcus pasteuri*, were identified using the VITEK 2 System. Initially, pure colonies were grown on blood agar after a 24-hour incubation at 37°C. These colonies were then transferred to sterile saline solution to prepare a bacterial suspension, the density of which was adjusted to be equivalent to a 0.5 McFarland standard. The bacterial suspension was then loaded onto a Gram-positive bacteria diagnostic card (GP card) and inserted into the VITEK system. The system incubated the card, read the biochemical changes resulting from bacterial growth, and compared them to its database to identify the bacterial species. After the analysis period, the system provided the final results identifying the bacterial isolates (Forbes et al., 2016).

Identification of resistance genes in whole-sequence bacteria: Microgen/Korea carried out whole genome sequencing analysis of the bacterial isolates. The short-read sequences were assembled as described previously. All the assembled genomes were aligned with all genes in the Refiner database using the BLAST software; genes that got the highest score were chosen as output results. A gene was deemed reportable if the genomic sequence covered at least 40% of the length of the resistance gene in the database. The identification of the most suitable genes was made following previously reported techniques. The percent of identical nucleotides between the detected sequence and the database resistance gene is known as the identity threshold (ID). The default identity threshold was set at 100%.

Results and discussion

Identification of bacterial isolates: In this study, 113 *Staphylococcus* bacterial isolates were obtained from various food sources and hand swabs randomly collected from different areas of Baghdad. These sources included spoiled cheese, fresh lamb and veal, and imported meat. Specifically, 35 isolates were obtained from spoiled salty cheese samples, 27 from spoiled sweet cheese samples, 18 from fresh local meat samples, 9 from imported meat samples, 2 from chicken shawarma, 3 from each of the grilled chicken samples, 2 from sausages, and 17 from hand swabs. The results showed that 75% of the isolates were fermenting mannitol, while 25% were not. The non-fermenting isolates were excluded from this study. The mannitol-fermenting isolates were cultured again on MSA medium and passaged multiple times to obtain pure isolates.

Antibiotic resistance testing to methicillin: In this study, 5 *Staphylococcus* isolates were found to be 100% resistant to disc methicillin. These isolates were isolated from meat and salted cheeses. The remaining 25 isolates had moderate sensitivity to the antibiotic methicillin, and the remaining 54 isolates were not resistant to methicillin and were excluded from this study.

VITEK Test: All five isolates identified as belonging to the *Staphylococcus* genus underwent the VITEK test for initial confirmation of the bacterial species and genus. The results

showed that four isolates identified as *Staphylococcus warneri,* isolated from meat and salted cheeses, had concordance rates of 99%, 98%, 98%, and 97%, respectively. One isolate from salted cheese was identified as *Staphylococcus haemolyticus* with a concordance rate of 96%. The isolate with the highest concordance rate was selected to continue the current study. Staphylococci are known to exhibit significant diversity and flexibility in metabolic expression, enabling them to adapt to varying environmental conditions and ensure survival. (Previous studies have shown that *Staphylococcus warneri* and *Staphylococcus pasteuri* are indistinguishable both culturally and microscopically due to their high degree of similarity. Therefore, identification can only be achieved using molecular methods (Bjorland et al., 2005).

Molecular characterization of mutations: The *Staphylococcus pasteuri* genome was assembled into 118 contigs with an average length of 23,879 base pairs (bp) and a total genome size of 2,647,116 bp, corresponding to an average quality genome assembly using the high-throughput sequencing techniques. Contigs are the short segments of the genome that are put together by computer and the number of Contigs is an indicator of the quality and completeness of the genome assembly. 2556 coding regions (CDS) were identified by genomic analysis. These are the areas that contain information for the production of proteins that enable the expression of genes and essential activity in the cell. This large number shows that there is high coding density, which means that the genome is very efficient in controlling protein production. A total of 8 rRNA genes, 72 tRNA genes and one tmRNA genes were found. They are crucial for the stability of protein translation and efficient protein synthesis in cells. The pattern of these genes is indicative of a sensitive balancing act between structural and regulatory components required for cellular function. The circular genomic map displays the general structure of the genome with coding sequences (CDS) on outer layers, and rRNA, tRNA and non-coding sequences on inner layers (Figure 1). Also included are key regulatory features, including transcription start sites (promoters), origin regions (Ori) and mobile genetic elements that are important for genome remodeling and evolutionary adaptation. The percentage of guanine to cytosine (GC) in the entire genome is a valuable indicator of the stability of DNA and the evolutionary history of a species of bacteria. GC skew is to determine skewness of G and C distributions in the two strands, which are used to determine the origin regions and direction of transcription of a gene. These markers are also important in comparative genomics and in the analysis of the structure of the bacterial chromosome. In general, the genome of S. *pasteuri* is well-organized reflecting high functional efficiency, and genetic adaptation and a robust basis for the understanding of the mechanisms of virulence and antibiotic resistance and the dynamics of horizontal gene transfer, with special interest in coagulase-negative staphylococci present in food environment (Abdullahi et al., 2025).

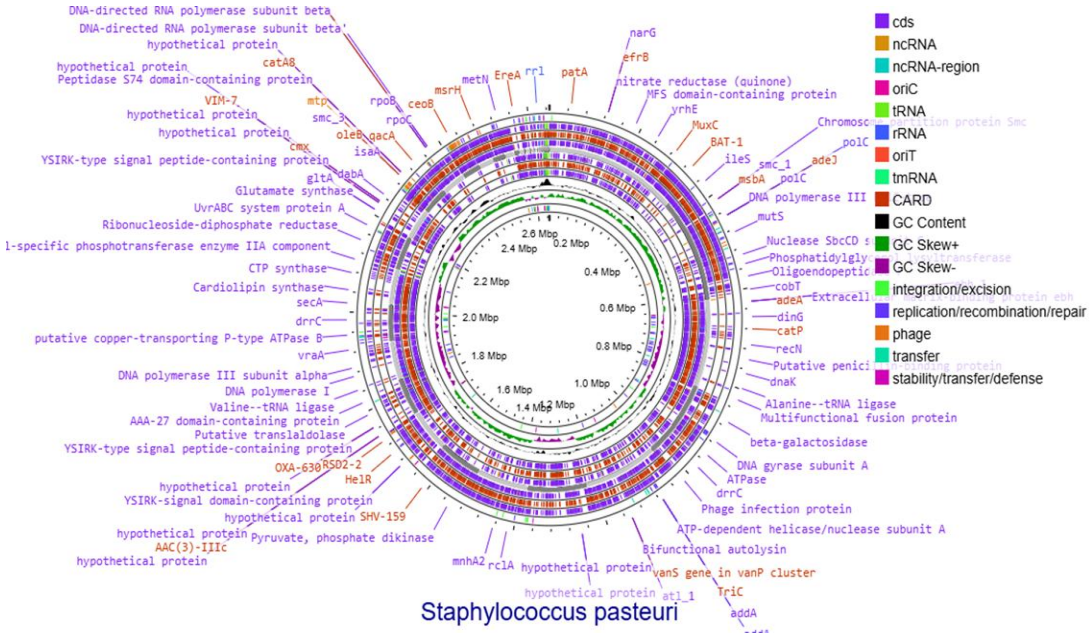

Figure 1. Circular genomic map of the *Staphylococcus pasteuri* isolate by using whole genome sequencing analysis

A complete genome of *Staphylococcus pasteuri* isolate is shown in Figure 2, along with the Single Nucleotide polymorphisms; SNPs density. The genome-wide distribution of mutations was studied using 10-kb sliding windows, a common method for the study of genome-wide genetic variation (Merda et al., 2024). The density of mutations within these 10-kb intervals was quantified across the gene locus to make it possible to identify trends in mutation distribution. The results revealed high variation in mutation density across regions, where mutation densities in some genomic regions were lower than in others. These variations were plotted as peaks and valleys which showed the variation in mutations in the genome. Single Nucleotide Polymorphisms (SNPs) are found in specific genomic regions, as shown by this distribution. There are some areas that have more mutations than others.

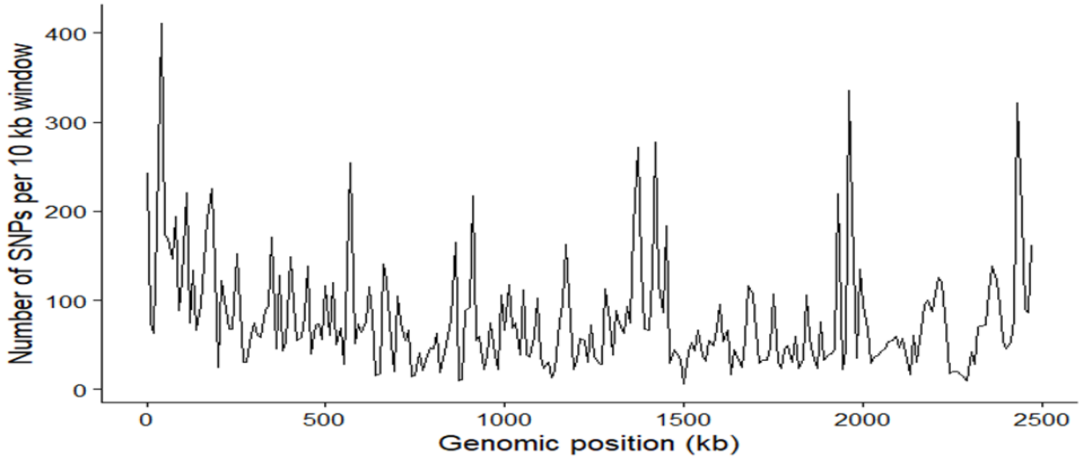

Figure 2. Mapping mutation density (SNP density) on the genome of the *Staphylococcus pasteuri* isolate

It was found that there was a significant difference in the level of SNP density in different regions of the genome, with the mutations being either low density (shown as a trough) or high density (shown as a peak). This heterogeneous distribution may result from a differing mutation rates or selective pressures and genetic recombination in specific genome (Sun et al., 2025; Connor et al., 2025). In addition, the genomic enrichment of SNAs in particular genomic regions suggests that these areas are (mutations hotspots) and could link to gene functions or adaptive evolutionary processes, as reported in recent comparative genomic studies using 10-kb windows to assess genetic diversity and pinpoint regions of selection (Huang et al., 2025).These results are in line with recent genomic analysis studies of SNPs in bacteria, including S. aureus, which revealed that the distribution of these SNPs is not random but instead clustered in mutation hotspot regions, which are frequently linked to genetic recombination or selective pressures (Merda et al., 2024; Connor et al., 2025). Other studies using whole-genome analysis (WGS) have confirmed that using 5–10 kb sliding windows is an effective method for detecting variations in mutation density and identifying genomic regions with high evolutionary activity (Zhang & Qian, 2025; Sun et al., 2025). Figure 3 illustrates the distribution of major gene mutations within different functions of the genome of the *Staphylococcus pasteuri* isolate. The results show a clear predominance of synonymous mutations, followed by Missense mutations, and then mutations located in adjacent gene regions. The high proportion of synonymous mutations is attributed to the variable nature of the genetic code. The presence of multiple codons encoding the same amino acid allows for changes in the DNA sequence without affecting the protein sequence, making these mutations mostly selectively neutral or near-neutral (Zhang & Qian, 2025). On the other hand, there are fewer high-impact variants, such as frameshift and stop-gain mutations, identified, as well as downstream mutations. This finding is expected in bacterial genomes because it is an indication of the nature of the genetic mutations that occur in the genome. These mutations often lead to drastic changes in protein structure or premature termination of translation, negatively impacting cell survival or viability. Consequently, these mutations are subject to purifying selection, resulting in less accumulation compared to mutations with limited impact. (Chu & Wei, 2020). Furthermore, this distribution reflects a common evolutionary pattern in organisms, where non-synonymous mutations are more likely to have detrimental effects due to their alteration of amino acid sequences, while synonymous mutations are indicative of the baseline mutation rate due to their relatively limited impac (Nei & Kumar, 2000). However, recent studies suggest that some synonymous mutations may not be entirely neutral, as they can affect messenger RNA (mRNA) stability or translation efficiency, reflecting the complex relationship between mutations and gene function (Rao et al., 2023). Accordingly, the pattern observed in this study is consistent with the general principles of molecular genetics and evolution, where the accumulation of mutations with limited effects prevails, while mutations with large effects are limited by natural selection. Results also showed the distribution of mutational burden across the genes most prone to mutation in the *Staphylococcus* pasteurei isolate by type of mutation (high, medium, low and modifying) as represented in Figure 4. The ebh gene has been shown to have a much higher number of mutations than the other genes, and there are more low- and medium-impact mutations.

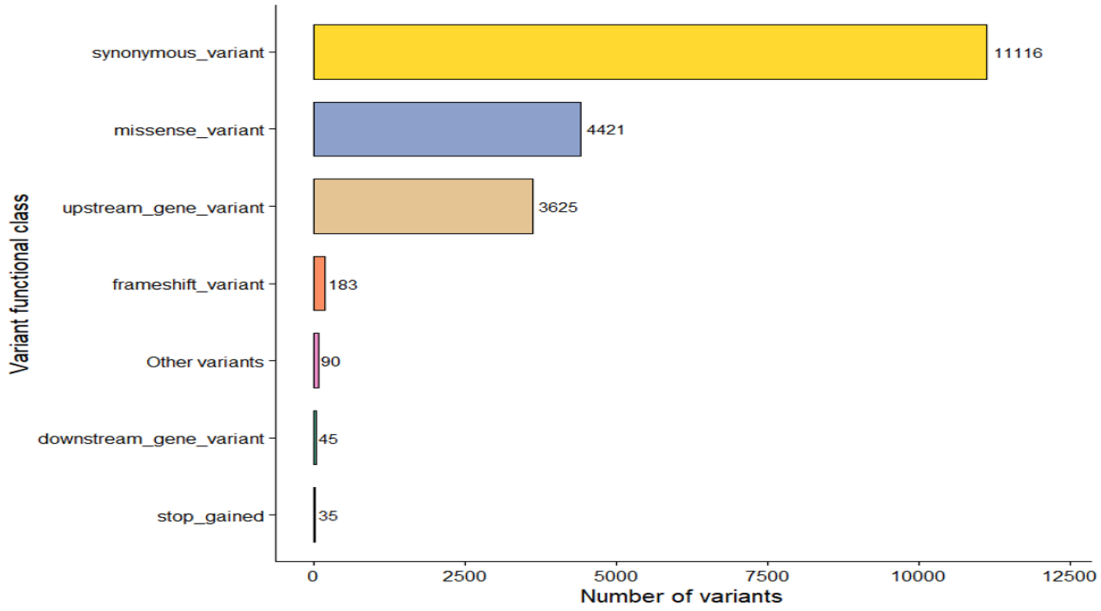

Figure 3. Functional distribution of gene mutations detected in the genome of *Staphylococcus pasteuri* isolate

This indicates considerable sequence variability of a large surface protein which is under environmental stress all the time. In the case of the ebh gene, which encodes extracellular adhesion proteins, the amount of mutation load could be associated with a gradual adaptive process to environmental dietary conditions or surface interactions. This can be explained by recent studies confirming that the *ebh* gene is one of the largest genes in the *Staphylococcus aureus* genome, encoding a large surface protein associated with the extracellular matrix and involved in environmental stress resistance and interaction with the host surface (Alves et al., 2024). Furthermore, recent genomic analyses have shown that this gene is classified among adaptation and adhesion-related genes, exhibiting a high degree of genetic plasticity across different clinical phenotypes (Girma, 2024). Accordingly, the marked increase in the mutational burden of the ebh gene in this study, with the predominance of low- and medium-impact mutations, reflects a pattern of adaptive microevolution, whereby bacteria retain the basic gene function while allowing molecular diversity that enhances their ability to adapt to different environments and ongoing environmental stresses. The results of the current study indicate that isolated *Staphylococcus pasteuri* exhibit a clear pattern of genetic variation, characterized by high mutation rates in regulatory genes such as blaR1 and stress- and metabolism-related genes such as trxB, galU, and isaB This pattern is consistent with many studies on coagulation-negative staphylococci (CoNS), which confirm that they represent an important genetic reservoir of resistance and environmental adaptation genes (Liu, 2009; Becker et al., 2014; Hiramatsu et al., 2013). Recent genomic studies have shown that CoNS, including *S. pasteuri*, are widespread in food and surface environments and exhibit high rates of antibiotic resistance and possess multiple resistance genes, reflecting their ability to survive in diverse environments (Kahraman Yılmaz & Berik., 2024) Analyses of prepared foods have also shown that these species are common food

contaminants, possessing resistance genes such as blaZ and mecA, as well as genes associated with multiple resistance, reinforcing their role as a genetic transfer source of bacterial resistance.((Byczkowska-Rostkowska et al., 2025). Concerning the blaR1 gene, recent evidence indicates that blaR1–blaI–blaZ systems exist in a fine regulatory network that regulates bacterial response to beta-lactams, and that alteration of this network is more likely to lead to a readjustment of the response level rather than to a total disruption of the system, which is an example of regulatory adaptation under continuous selective pressure (Jiang et al., 2024). This is in line with results from this study that showed intermediate-effect mutations to be dominant in blaR1. Recent studies on CoNS have also shown that this bacterium possesses a high capacity for environmental adaptation through the accumulation of mutations in genes associated with oxidative stress and metabolism, including thioredoxin and cell wall genes, which contributes to enhanced survival under unstable environmental conditions (Abdullahi et al., 2025). Specifically, *S. pasteuri* exhibits the ability to thrive in food and environmental surfaces, with evidence of resistance to several antibiotics, such as β-lactams and macrolides, highlighting its high genetic plasticity (Kahraman Yılmaz & Berik., 2024). Therefore, the mutational pattern observed in this study does not represent functional loss, but rather reflects an adaptive microevolution process, *S. pasteuri* is reorganizing its response to antibiotics and environmental stress through changes in regulatory and metabolic genes, enhancing its ability to survive in diverse food and surface environments. The overall low proportion of high-effect mutations as compared to low- and medium-effect support the notion that the isolate is undergoing purifying selection, with localized evolutionary hotspots instead of widespread genomic evolution. Recent studies have shown that staphylococci isolated from food, including CoNSs such as *S. pasteuri,* exhibit genetic diversity primarily in resistance, adhesion, and regulatory genes, while core genes remain more stable due to ongoing purifying selection that maintains the bacterium's vital functions (Wang et al., 2019). Genomic analyses of CoNS isolate from food (such as meat, processed foods, and fish) have confirmed that these bacteria act as gene reservoirs for resistance genes, with the spread of mobile genetic elements and genes such as blaZ and *mecA*, reflecting the presence of non-clinical selective pressure leading to localized changes in the genome rather than widespread destructive mutations (Byczkowska-Rostkowska et al., 2025). This observation suggests more focused systems-level evolutionary divergence of adhesion, regulatory and resistance genes in the isolate Locally Isolated *Staphylococcus pasteuri* from food than in the basic functional backbone of most proteins (or basic functional vectors), which is more plausible in a food-adapted/environment-conditioned isolate than it would be amongst isolates derived from hospital patients under direct antibiotic selection pressure. As Figure 5 shows the distribution of high-impact variants in functional genes of the isolate *S. pasteuri*, showing a selective evolutionary process targeted towards genes related to virulence, gene transfer, molecular organization, and cell wall structure, and not a random distribution of mutations in the genome. It was also noticed that the gene with the highest mutations belongs to this virulence protein, SAV1978, of the virulence-associated passenger proteins. The saturation of the highest number of HIGH mutations in this gene suggests a putative functional change regarding adhesion or surface interaction, which may be a reflection that the isolates are adapting to their environment or being pressured by it (Karlsson et al., 2024).

This data may indicate that the isolate is undergoing a modification stage in its surface components, a known trait in bacteria that have been under long-term environmental selection (Abdullahi et al., 2025). Genes related to gene transfer, such as Mobilization protein and Relaxes/mobilization nuclease, also show a high number of high-impact mutations. Potentially signaling activity or reconfiguration in plasmid or quasi-plasmid components, this could point to dynamics in the horizontal gene transfer system (Smillie et al., 2010).

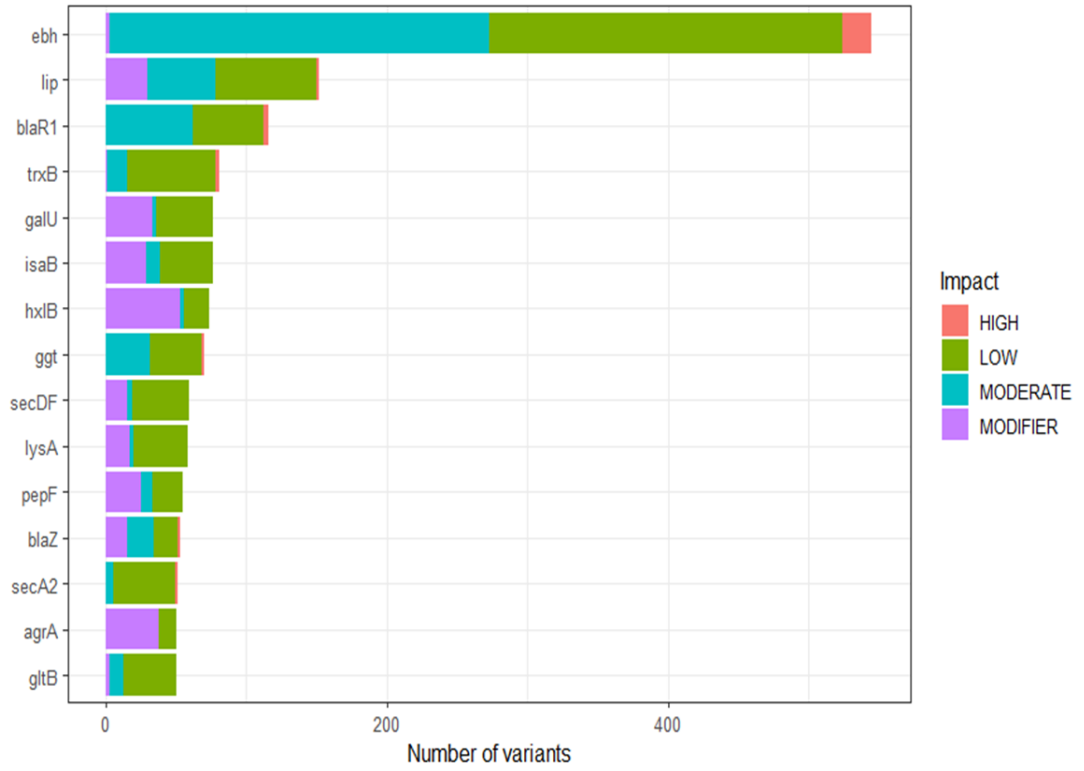

Figure 4. The mutational burden distribution in the most frequently mutated genes in the *Staphylococcus pasteuri* isolate, categorized according to the type of mutation (High, Moderate, Low, Modifier)

The capacity of an isolate to transmit genetic material is an important epidemiological indicator, and high-impact mutations in these genes may affect the control or efficiency of transfer (Dündar & Çakırlar, 2025). In similar studies on *Staphylococcus pasteuri* isolated from seafood, these bacteria were found to carry multiple resistance patterns with clear differences between isolates in resistance genes, suggesting that the genetic changes in them are localized rather than systemic, and reflect a gradual environmental adaptation rather than an acute pathogenic evolution (Kahraman Yılmaz & Berik., 2024). Mutations in the restriction-modification system type I gene suggest a possible reconfiguration of the defense mechanism against foreign DNA (Loenen et al., 2014). The results of our current study are consistent with the findings of previous research (Vasu & Nagaraja, 2013) confirming that restriction systems are among the most important barriers to preventing the entry of foreign DNA, while simultaneously representing dynamic evolutionary elements that control gene flow and horizontal transfer within bacteria. Recent studies have shown that these systems not only function as "bacterial immunity" but also play a direct role in

shaping genomic evolution by regulating the entry of plasmids and mobile genetic element (Agapov et al., 2024). Alterations in these systems may increase genomic plasticity and allow for the selection of new genetic elements under environmental pressure (Kang et al., 2025). Implementing analyses of bacterial genomes has also demonstrated that restriction-modification (R-M) systems are one of the most important obstacles to the transfer of plasmids from strain to strain and thus leave a clear "evolutionary signature" on the distribution of genes within bacteria. This implies that changes in these systems will directly affect the dynamics of horizontal gene transfer and environmental adaptation (Shaw et al., 2023). Moreover, such genomic rearrangements or translocations have been reported and this suggests that these are active, not static, evolutionary elements (Furuta & Kobayashi, 2022). The results also indicated mutations in the enzymes involved in the peptidoglycan synthesis pathway, such as the alanine racemase and trans glycosylase enzymes that are required in the formation of the cell wall. Recent research has shown that these enzymes are among the proteins most susceptible to selective pressure; any alteration in them can result in a change in the rigidity or structure of the cell wall, consequently impacting on the ability of the bacteria to resist environmental factors or on the ability of antibiotics to affect the cell wall (Zhao et al., 2025). Recent studies confirm that bacteria use complex regulatory networks to respond to oxidative stress, where different gene sets are dynamically (pulsatile or gradual) activated to ensure protection and survival without affecting essential functions (Choudhary et al., 2024). This indicates that changes in transcriptional regulators such as MerR lead to reprogramming of gene expression rather than a direct change in resistance**.** Furthermore, genomic analysis reveals that the presence of metabolic genes, such as NAD+-dependent epimerase/dehydrates enzymes, along with components of the phosphoinolymphate transport system (PTS), particularly the IIC subunit, reflects the metabolic adaptability of *Staphylococcus pasteuri* to diverse carbon sources in various nutrient and environmental settings. Recent genomic studies of *Staphylococcus* species have shown that carbon metabolism systems, including PTS, play a pivotal role in promoting survival and environmental adaptation, especially in food-associated isolates and non-clinical environments (Hamza et al., 2024). Furthermore, the widespread presence of resistance genes and the ability to utilize diverse nutrient resources reflect a high degree of genomic flexibility in this bacterial genus (Jiang et al., 2024). In a study conducted on *Staphylococcus pasteuri* strains from food (mussels), Kahraman Yılmaz & Berik., (2024) found that these bacteria were equipped with a series of functional genes that are linked to their survival in food environments, including multiple antibiotic resistance genes including blaTEM, strA-strB and aphAI-IAB, which suggest a high level of adaptability to environmental stresses and antibiotics. These results are consistent with the notion that metabolic and membrane transport systems (like PTS) cooperate with resistance genes to help bacteria survive in the complex environment. On the other hand, the presence of genes containing uncharacterized domains (DUFs), such as DUF3310 and DUF771, reflects the existence of genomic components that are not fully understood. However, recent genomic studies of *Staphylococcus* aureus have shown that many of these previously unknown genes are often associated with mobile genetic elements (MGEs) or with regulatory and stress response mechanisms, which play an important role in bacterial adaptation and evolution (Ramos &

Cunha., 2024). Furthermore, the detection of high-impact mutations in these genes, as seen in genomic mutation studies, is often linked to functional changes affecting gene regulation or antibiotic resistance (Gómez-Casanova et al., 2024). Therefore, the combination of Metabolic genes (such as NAD+ and PTS enzymes), unknown genes (DUFs), and high-impact mutations strongly suggests the presence of a sophisticated adaptive system in *Staphylococcus pasteuri* that enables it to survive in nutrient-dense and antibiotic-stressed environments, potentially with as-yet-undiscovered regulatory functions.

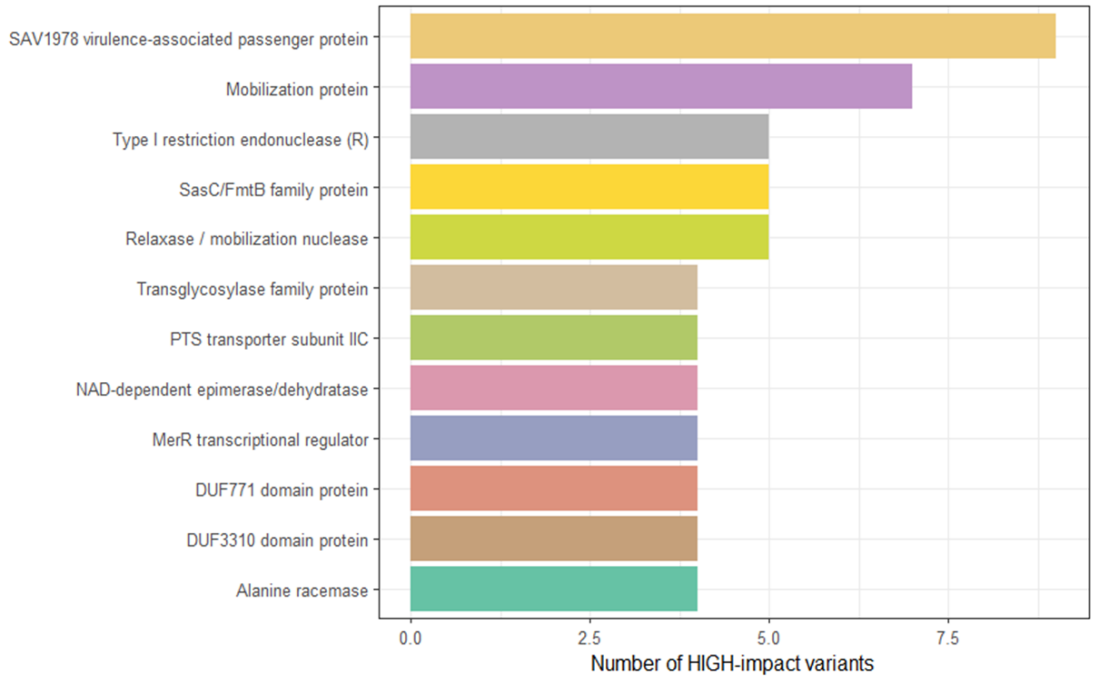

Figure 5. shows the distribution of high-impact variants in functional genes of the isolate *S. pasteuri*

Conclusion: *Staphylococcus pasteuri* is a widespread and resilient component of the food microbiome. This bacterium is often recovered from diverse matrices such as dairy, meat, and fish products. Results from whole-genome sequencing and analysis of genetic variance (ANOVA) indicate that single nucleotide polymorphisms (SNPs) have a non-random distribution across its genome. In its genome, mutations are highly concentrated at local evolutionary hotspots. While, key critical regions are highly conserved under purifying selection. Among these, the ebh gene acts as a major locus for adaptive microevolution. This gene accumulates a significant load of low- and moderate-effect mutations. These drive sequence diversity in its encoded cell surface adhesion to cope with ongoing environmental and matrix-associated stresses. The results show that this targeted genetic variation is mostly reflected in critical regulatory networks (blaR1) and stress- or metabolism-related pathways (trxB, galU, isaB). This indicates a precise metabolic and regulatory rearrangement rather than a functional collapse. A selective inhibition of high-impact mutations in functional genes governing virulence (SAV1978) was designed for growth in non-clinical, nutrient-rich food environments. Ultimately, this selective inhibition, which highlights horizontal gene transfer (mobilizer proteins), cell wall synthesis (alanine racemase) and defense

mechanics (type I restriction-modification), is a complex evolutionary adaptation at the systems level.

Author contributions

Zahraa A. Ahmed conducted the lab experiments, data collection and analysis, wrote the manuscript and participated in development of the study concept. The research work was supervised by Asmaa S. Ahmed and Huda M. Mahmood who were also involved in data analysis. The final version of the manuscript was read, commented on, and approved by all of the authors.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethical considerations

Throughout the study all ethical standards and guidelines were adhered to. The authors certify that there is no fabrication, falsification, plagiarism or other scientific misconduct. The study was ethically approved by institutional ethics committee at University of Baghdad, College of Agricultural Engineering Sciences (2026).

Conflict of interest

There is no conflict in this study.

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ویژگی‌یابی مولکولی جهش‌ها در *Staphylococcus pasteuri* کوآگولاز منفی مقاوم به متی‌سیلین جداشده از نمونه‌های مختلف مواد غذایی

زهراء ا. احمد

گروه زیست‌فناوری، دانشکده علوم، دانشگاه الانبار، رمادی، عراق. ایمیل: zahraaabdahmed@uoanbar.edu.iq

اسماء س. احمد

دانشکده علوم مهندسی کشاورزی، دانشگاه بغداد، بغداد، عراق. ایمیل: asmaa.s@coagri.uobghdad.edu.iq

هدی م. محمود

* *نویسنده مسئول*. گروه زیست‌فناوری، دانشکده علوم، دانشگاه الانبار، رمادی، عراق. ایمیل: huda.mahmood@uoanbar.edu.iq

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چکیده

هدف: استافیلوکوک‌ها به‌طور طبیعی در بدن انسان و حیوانات، به‌ویژه در دستگاه‌های گوارش و تنفس، حضور دارند. گونه‌های *Staphylococcus* از جمله میکروارگانیسم‌های رایج در محیط‌های مرتبط با مواد غذایی هستند و از محصولات متنوعی نظیر گوشت، فرآورده‌های لبنی و غذاهای آماده مصرف جداسازی شده‌اند. هدف این مطالعه، جداسازی و شناسایی *Staphylococcus pasteuri* از انواع فرآورده‌های غذایی (گوشت و پنیر) و همچنین از دست‌اندرکاران تهیه و عرضه مواد غذایی، و بررسی الگوهای مقاومت آنتی‌بیوتیکی و جهش‌های ژنتیکی آن‌ها با استفاده از داده‌های توالی‌یابی کل ژنوم بود.

مواد و روش‌ها: در مجموع ۵۸ نمونه مواد غذایی شامل گوشت تازه، گوشت پخته و پنیر، به همراه ۱۳ نمونه سواب دست از کارکنان تهیه و عرضه مواد غذایی، از شهر بغداد، عراق، جمع‌آوری شد. برای غربالگری مقاومت به متی‌سیلین از روش انتشار دیسک کربی–باوئر (Kirby–Bauer) استفاده شد و نتایج آن با سیستم خودکار VITEK 2 تأیید گردید. تعدادی از سویه‌های مقاوم برای توالی‌یابی کل ژنوم (Whole-Genome Sequencing; WGS) با استفاده از فناوری Illumina انتخاب شدند تا ژن‌های مقاومت و پلی‌مورفیسم‌های تک‌نوکلئوتیدی (Single-Nucleotide Polymorphisms; SNPs) مورد بررسی قرار گیرند.

نتایج: *Staphylococcus pasteuri* با موفقیت از نمونه‌های غذایی و نیز از دست‌اندرکاران تهیه مواد غذایی جداسازی شد و نتایج، احتمال انتقال این باکتری از طریق زنجیره غذایی را نشان داد. بررسی‌های فنوتیپی و مولکولی، شیوع بالای ویژگی‌های

مقاومت به چند دارو را در جدایه‌ها آشکار ساخت. تحلیل توالی‌یابی کل ژنوم (WGS) وجود مسیرهای متابولیکی مهم، از جمله آنزیم‌های وابسته به NAD^+ و سامانه انتقال فسفوانول‌پیرووات فسفوترانسفراز (Phosphoenolpyruvate Phosphotransferase System; PTS) را نشان داد. این یافته‌ها بیانگر توان متابولیکی بالا و قابلیت سازگاری گسترده این باکتری با انواع مختلف محیط‌های غذایی است. همچنین، تحلیل ژنومی انعطاف‌پذیری ژنتیکی قابل توجهی را نشان داد که با حضور عناصر ژنتیکی متحرک، تعیین‌کننده‌های مقاومت آنتی‌بیوتیکی و تعداد زیادی پلی‌مورفیسم تک‌نوکلئوتیدی (SNP) همراه بود. از جمله یافته‌های مهم، شناسایی جهش‌های با اثر زیاد در دومین‌های با عملکرد ناشناخته (Domains of Unknown Function; DUFs)، به‌ویژه DUF3310 و DUF771 بود که احتمالاً دارای نقش‌های تنظیمی جدید هستند.

نتیجه‌گیری: نتایج این مطالعه نشان داد که *Staphylococcus pasteuri* دارای ساختار ژنومی بسیار انعطاف‌پذیر و پیچیده‌ای است که به آن امکان بقا، سازگاری با محیط‌های مختلف و انتشار ژن‌های مقاومت آنتی‌بیوتیکی در زنجیره غذایی را می‌دهد. این ویژگی‌ها، *S. pasteuri* را به یک نگرانی مهم در حوزه سلامت عمومی و ایمنی مواد غذایی تبدیل می‌کند و ضرورت پایش و کنترل آن را بیش از پیش آشکار می‌سازد.

کلمات کلیدی: ایمنی مواد غذایی، پلی‌مورفیسم تک‌نوکلئوتیدی (SNP)، توالی‌یابی کل ژنوم (WGS)، مقاومت به متی‌سیلین، *Staphylococcus pasteuri*

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