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ORIGINAL RESEARCH

EVALUATIONG THE ROLE OF PHARYNGITIS-CAUSING STAPHYLOCOCCUS AUREUS IN PROMATING INFLAMMATION IN DIFFERENT IMMUNE CELLS

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ABSTRACT

Background: Pharyngitis is a common upper respiratory infection that primarily affects children and adolescents. Although Staphylococcus aureus is not considered a primary etiological agent, its role in chronic and recurrent pharyngitis remains clinically relevant and underexplored.
Objective. This study aimed to assess the prevalence, antibiotic resistance, and virulence profile of S. aureus in pharyngitis cases, and to evaluate its inflammatory impact on host immune cells.
Materials and Methods:A total of 122 throat swabs were collected from patients aged 4 to 17 years presenting with pharyngitis across various healthcare centers. Patients were categorized into acute and chronic groups. S. aureus was identified through morphological characteristics and biochemical assays (coagulase, DNase, mannitol fermentation), with confirmation via the VITEK 2 system. Antimicrobial susceptibility was assessed using the disk diffusion method. PCR analysis was conducted to detect the presence of mecA, femA, and LukE virulence genes. Furthermore, splenocytes and lymph node cells were exposed to killed S. aureus, and the expression of inflammatory genes (NF-κB, IL-6, TNF-α) was evaluated using RT-qPCR.
Results:S. aureus was detected in 70 out of 122 samples (57.3%). The isolates showed high resistance rates to oxacillin (100%), amoxicillin (91%), vancomycin (85.7%), and ceftriaxone (80%), while resistance to levofloxacin and clindamycin was lower (7.1% and 5.7%, respectively). Multidrug resistance (MDR) was observed in 5.7% of isolates. PCR confirmed the presence of mecA, femA, and LukE genes in several MDR strains. Immune cell exposure to killed S. aureus resulted in significant upregulation of NF-κB, IL-6, and TNF-α, indicating a pronounced pro-inflammatory effect.
Conclusion: The study highlights the potential contribution of Staphylococcus aureus to pharyngeal inflammation, particularly in persistent or recurrent infections. Its high resistance rates and capacity to induce immune activation emphasize the need for targeted diagnostic and therapeutic strategies in managing pharyngitis

Keywords: pharyngitis, femA, TNF-α, RT-qPCR, IL-6, mecA, Luk E, gene expression.

INTRODUCTION

Pharyngitis is caused by inflammation of the oropharyngeal mucous membranes[1]. It is more common in children aged between 5-15. Infections are more likely to occur in the winter and early spring, it is unknown exactly how cold exposure impacts haryngitis seasonality. Seasonality has been explained by a variety of theories, including changes in host immunity over time, pathogen virulence, human behavior, and variations in relative humidity and ambient temperature [2].

Direct or indirect contact with an infected individual can spread this disease through contaminated food and items, big respiratory secretions, or droplets [3]. The rates of bacterial isolation and bacterial pharyngitis infection transmission are significantly higher in densely populated and closed communities. According to estimates, there may be 30 million children in underdeveloped nations that suffer from pharyngitis, whereas only 1.5 million in

industrialized countries[4]. The clinical symptoms include throat pain, fever, chills, headache, abdominal pain, nausea, and vomiting. Swollen, painful anterior cervical lymph nodes are a common feature, especially in younger children [5]. This illness is frequently caused by bacteria, including Group A streptococcus, Staphylococcus aureus, Pseudomonas aeruginosa, Chlamydia pneumoniae, Klebsiella pneumoniae, Streptococcus pneumoniae, and Diphtheria[6]. Allergies, malignancy, trauma, reflux, and specific toxins are some of the less frequent causes of pharyngitis[7].

Staphylococcus aureus is a prominent opportunistic pathogen known for its ability to cause a broad range of infections. It carries numerous virulence-associated genes, which are located either on its chromosome or on mobile genetic elements. These virulence factors play critical roles in enabling the bacterium to colonize host tissues, invade mucosal surfaces and injured skin, spread systemically, and evade host immune responses [8]. The pathogenic potential of Staphylococcus aureus is influenced by a variety of surface-associated structures, including capsular polysaccharides and protein A, as well as secreted factors like coagulase, enterotoxins, hemolysins, exfoliative toxins, and the toxic shock syndrome toxin. Additionally, genetic elements like the mecA gene and Panton–Valentine leukocidin (PVL) contribute to its virulence. Nonetheless, determining the specific contribution of individual virulence components to the overall infectious process remains challenging [9]. Depending on the organism producing the pharyngitis, the infection mechanism will vary. In cases of bacterial pharyngitis, Staphylococcus aureus immediately invades the pharyngeal mucosa [4]. Extracellular components like proteases aid in this invasion, which results in inflammation that produces fever, edema, exudates, and difficulty swallowing. In a broader sense, the inflammation can also exacerbate symptoms of otitis and sinusitis, even in cases when the same bacteria have not affected those regions [10].

Through the activation of the inflammasome and the recruitment of neutrophils, Staphylococcus aureus causes inflammation. These reactions are essential for pathogens to destroy, but are linked to significant tissue damage. Therefore, this inflammation must be controlled to avoid harming the host tissue. Today, tissue culture is a commonly used method to investigate how bacterial infections interact with host cells. It allows researchers to study how cells grow and behave in a controlled environment. It can be applied to cell signaling, cell differentiation, gene expression, and other areas [11]. Therefore, the genetic information included in a gene or nucleotide sequence can be used to control gene expression, which in turn controls the structure and production of proteins in a cell [12]. Thus, it is possible to examine the differences between normal and infected cell pathways by determining the biological pathways and gene expression levels linked to the genes implicated in the disease, and this leads to identifying the genetic basis of the defective pathway [13].

MATERIALS AND METHODS

Source of sample:

Between January and July 2023, clinical sampling was carried out in Tikrit City across multiple healthcare settings, including Salah Al-Din General Hospital, Tikrit Teaching Hospital, and various private clinics. A total of 122 throat swab specimens were obtained from individuals clinically diagnosed with pharyngitis. These patients exhibited a medical history marked by tonsillar hyperplasia and/or recurrent episodes of tonsillitis, often following failed courses of antibiotic therapy. Additionally, all pertinent data were documented for each patient, including address, age, sex, underlying medical conditions, and other details.

S. aureus isolation and identification

Initial culturing of the clinical samples was performed on blood agar, nutrient agar, and MacConkey agar, and incubation at 37°C for 24 hrs. Subsequent colony growth was subcultured onto selective and differential media, including Mannitol Salt Agar as described by Brown et al. [(14)]. Identification of Staphylococcus aureus was based on colony morphology and key biochemical tests—namely, catalase, coagulase, and mannitol fermentation according to CLSI guidelines. Final confirmation of the isolates was achieved using the VITEK 2 automated system (bioMérieux, France), adhering to the manufacturer's standardized procedures.

Antibiotic Susceptibility Test

The antimicrobial susceptibility of the isolates was assessed using the disk diffusion technique on Mueller-Hinton agar, in accordance with the standards outlined by the Clinical and Laboratory Standards Institute (CLSI) [15]. In summary, the suspension was inoculated on a Mueller-Hinton agar plate using sterile swabs following comparison with a 0.5 McFarland standard solution. The antibiotic discs used in this study are as follows: Oxacillin, Penicillin G, Ceftriaxone, Amoxicillin, Vancomycin, Azithromycin, Clindamycin, Gentamycin, Levofloxacin, and chloramphenicol. Thereafter, the plate

was incubated for 24 hrs at 37°C. Inhibition zone measurements were then conducted and compared to the standard levels outlined in the CLSI documentation[16].

Genotypic identification of mecA, femA and lukE genes

DNA was extracted using a ready kit (Tinzyme, China) from overnight cultures of S. aureus isolates. The purity of the extracted DNA was visually assessed using a Nanodrop Spectrophotometer set to 260/280 nm and horizontal gel electrophoresis in 1% agarose. A total volume of 20 µL was used for the PCR reactions to detect the mecA, femA, and lukE genes. A total reaction volume was composed of 10 µL Master Mix, 3 µL DNA template, 1 µL of each primer (forward and reverse), and 5 µL nuclease-free water.. Following the procedure of García et al. [(17)], PCR was used to detect mecA, and Kobayashi et al. [(18)], a method for detecting the femA gene. The following primer sequences were used in this investigation to detect the mecA, femA, and lukE genes: Table 1.
anterior face (p = 0.000), more obtuse cranial base angle (p = 0.002) and longer anterior cranial base (SN length p = 0.000 as well). These characteristics indicate that the craniofacial morphology of mothers could be larger in vertical features and protuberance towards the anteriors in the cleft affiliated cases. Further significant differences were also observed when parental pair fathers contrasted against control fathers. These comprised the smaller area of symphysis (p = 0.047), a shorter length of palate (p = 0.003) and a much smaller area of maxillary region (p = 0.027), with a longer SN measures (p = 0.018) and a more acute cranial base angle (p = 0.016). These differences support the hypothesis that smaller midface and mandibular measurements can be phenotypic predictors of susceptibility to clefts in parental pair mothers their total mandibular length was longer (p = 0.000), their anterior facial height was greater (p = 0.005), their SN length was longer (p = 0.000) and the angle components of their cranial base were sharper (p = 0.000). It is also important to note that the length of the clivus (S-Ba) was significantly longer among these mothers (p = 0.000), and this fact can be related to the structural defects of the cranial base that also leads to a risk of developing clefts. Among the five powerful discriminants between cleft and non cleft groups included in the multivariate analysis by use of the Mahalanobis distance was the five cephalometric variables in fathers (total mandibular area, cranial base angle, palatal length, SN length and symphysis area). In mothers the total mandibular length and cranial base angle were the most important. These discriminant function analysis the existence of sexually dimorphic patterns in cephalometric features relative to cleft risk (Table 1-5).

Table 1. Sequence of oligonucleotide primers for mec A, fem A and lukE genes

Primer name	Primer sequence		Annealing Temp. (°C)	Amblicon size (bp)	References
mecA	F	TCACCAGGTTCAAC[Y]CAAAA	53	356 bp	(18)
	R	CCTGAATC[W]GCTAATAATATTTC			
femA	F	AGACAAATAGGAGTAATGAT	48	509 bp	(17)
	R	AAATCTAACACTGAGTGATA			
lukE	F	TTGTCAGTAGGACTGATTGCACCTTTAGC	62	906bp	(51)
	R	TTAATTATGTCCTTTCACTTTAATTTCGTGTGTT			

The thermocycler (Applied Biosystem/USA) was used to perform PCR conditions for the amplification of the mecA, femA, and lukE genes following the guidelines from earlier research. The PCR results were recorded after being electrophoresed for 90 minutes at 5 V/cm in a 2% agarose gel. The Gel Documentation System was used to image the gel after it had been stained for 45–60 min by immersing it in ethidium bromide while stirring.

Preparation of dead bacterial cells

Colonies of growing S. aureus were extracted using a disposable loop from a particular culture medium. After being combined with two mg/ml of gentamicin, the bacteria were cultured for three hours at 37°C to kill them. The bacteria were collected and kept at 4°C after being centrifuged for 5 min at 12,000 rpm. They were then resuspended in full medium. As required, the bacterial suspension was diluted [19].

Mice

We used 8–10 week old (Mus musculus) mice for all investigations. It was purchased from the house of Laboratory Animals at the University of Mosul's College of Veterinary Medicine.

Preparing the complete culture medium

The complete culture medium was prepared by combining 9 mL of RPMI with 1 mL of fetal bovine serum (FBS) and 0.5 mL of a penicillin-streptomycin solution. The mixture was thoroughly homogenized before application.

Preparation and isolation of the immune cells

A specialist veterinarian dissected immune cells, which included splenocytes and lymph nodes, to collect them. Spleen cells were isolated following the aseptic euthanasia of the mice. In short, splenocytes were cultivated in full media following the removal of erythrocytes using red blood cell (RBC) lysis solution. The tissue was broken up using a Stomacher blender to create single-cell suspensions, and the cells were then rinsed twice with PBS for five minutes at 3000 rpm in a full medium. After that, the cells were planted with 500,000 cells per well in a 96-well plate [20]. Mesenteric Lymph Node (MLN) Isolation: Lymph nodes were extracted from killed mice as described by Qasim[21]. In short, a Stomacher Blender was utilized to break down tissue into individual cells in a full medium. Following this, the cell suspension was passed through a 70 µm filter and subsequently centrifuged at 1300 rpm for 10 minutes. The cells were quantified using an automated cell counting system after resuspension of the pellet in complete medium.

Preparation Procedure of Specimen

Following independent homogenization of the lymph nodes and spleen, the resulting products were placed in separate tubes containing five milliliters of the preservation media. The samples were first centrifuged at 1300 rpm for 7 min. After carefully discarding the supernatant, 600 µL of red blood cell lysis buffer was added to the pellet and gently mixed for one minute. The resulting suspension was then passed through a specific filter. The filtrate was subsequently centrifuged again at 1300 rpm for 10 min, and the supernatant was removed. One milliliter of complete medium was added to the cell pellet, and approximately 200 µL of this mixture was distributed into each well. In addition, 12.5 µL of heat-killed bacteria was added to three designated wells. The culture plate was incubated in a CO_2 incubator for 48 hours. After incubation, the contents of each well were transferred into individual Eppendorf tubes and centrifuged at 10,000 rpm for 10 min. Following removal of the supernatant, 700 µL of TRIzol reagent was added to the pellet, which was then stored at −80 °C for subsequent RNA extraction and RT-qPCR analysis.

Molecular Detection

The mRNA expression of related genes in treated and untreated samples was investigated using real-time quantitative PCR (qPCR) with cDNA generated from each specimen's total RNA. The gene expression was then evaluated using the Triazole Up Plus RNA Kit from the transgenebiotech firm. Table 1 lists the specific primers that were utilized.

RNA Extraction and cDNA Synthesis

Total RNA was isolated from frozen specimens using the TransZol Up Plus RNA Kit (TriReagent-based, TransGen Biotech). Elution was carried out in 20 µL of RNase-free water, with the volume adjusted based on the amount of precipitated RNA. RNA yield and purity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). For complementary DNA synthesis, 500 ng of total RNA were reverse transcribed using the First-Strand cDNA Synthesis SuperMix kit (Beijing, China), in accordance with the manufacturer's protocol.

Real-Time Quantitative PCR (RT-qPCR)

Quantitative real-time PCR (qRT-PCR) was conducted using the TransStart Green qPCR SuperMix, a pre-formulated reaction mix that includes dNTPs, PCR enhancers, stabilizing agents, and TransStart Taq DNA Polymerase combined with SYBR Green dye. Gene expression analysis was performed by comparing target genes from both control and treatment groups relative to a housekeeping gene. Amplification reactions were carried out on the LightCycler 480 system following the protocol recommended by the manufacturer. Thermal cycling conditions consisted of an initial denaturation at 98°C for 30 seconds, followed by 45 cycles of 98°C for 10 seconds and 60°C for 30 seconds. All reactions were run in triplicate, and the relative mRNA expression levels were normalized against the reference gene using the ΔCt method [13].

Table 2. provides the specific details of the primer sequences used for RT-qPCR

Gene	Primer	Sequence	References
GAPDH Housekeeping	F	AGGTCGGTGTGAACGGATTTG	(22)
	R	TGTAGACCATGTAGTTGAGGTCA	
TNF	F	GCCTCTTCTCATTCCTGCTTG	(50)
	R	CTGATGAGAGGGAGGCCATT	
IL-6	F	CGTGCGTGACATCAAAGAGAA	(50)
	R	TGGATGCCACAGGATTCCAT	
NF-KB	F	CGCAAAAGGACCTACGAGAC	(23)
	R	TGGGGGAAAACTCATCAAAG	

3.RESULTS

Inpatients from Tikrit Teaching Hospital, Salah Al-din General Hospital, private clinics, and medical institutions in Tikrit city provided study samples.

Identifying and Isolating Staphylococcus aureus

The bacteriological investigation showed that 70 clinical Staphylococcus aureus isolates were obtained from throat swabs based on the clinical diagnosis, the bacterial cultures, and the results of biochemical tests. This has been confirmed using the VITEK 2 system.

Distribution of study samples according to gender and Age

Males and females experienced pharyngitis at varying rates. The gender distribution revealed that 54.2% (n.38/70) of the children diagnosed with pharyngitis were male, while 45.7% (n.32/70) were female. The most commonly infected age group was 5 - 10 years, representing 37 (52.8%), followed by the age group from 10 - 15, and the age group 1 - 5 was less infected by S. aureus, 3(4.2%), as shown in Figure 1.

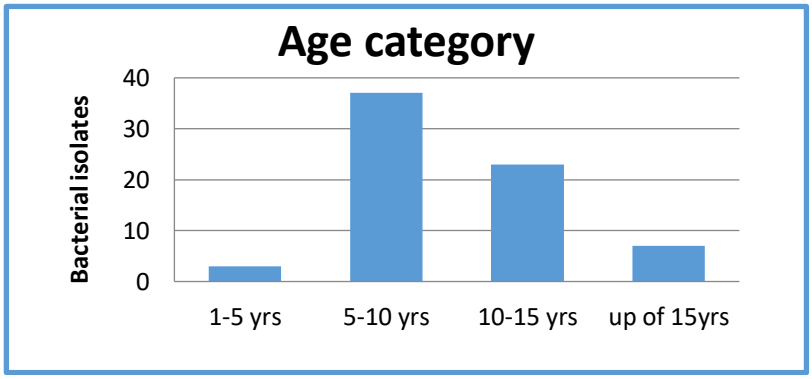

Figure 1. The distribution of Staph. aureus isolates according to the age groups.

The distribution according to the kind of infection

Pharyngitis patients are divided into two types with different numbers and proportions; the highest percentage of patients were with chronic pharyngitis, 55.7 % (n. 68/122), followed by acute pharyngitis, 44.2 % (n. 54/122), as shown in Figure 2.

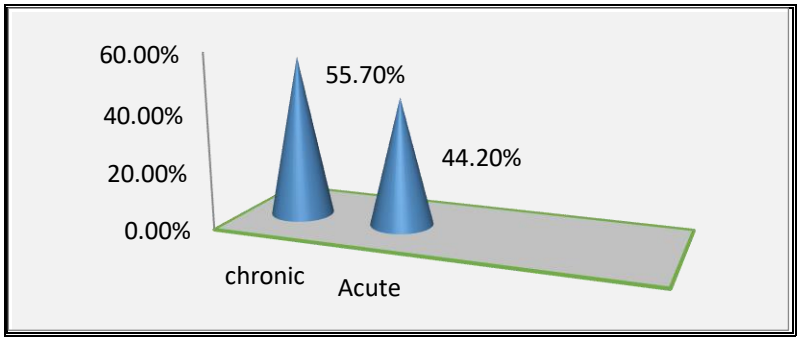

Figure 2. Showed distribution infection into chronic and acute

Antimicrobial susceptibility test

This study used the disc diffusion method to examine the antibacterial profile of 70 Staph. aureus isolates. 100% of the samples had a high level of resistance to oxacillin, with Amoxicillin (91%), Vancomycin (85.7%), Ceftriaxone (80%), and penicillin G (40%) following closely behind. In contrast, the resistance rate to Azithromycin, Gentamicin, and Chloramphenicol was 32 percent, 31 percent, 7.1%, Levofloxacin, and 5.7%, respectively.

Genetic detection of Virulence factors of Staphylococcus aureus by PCR

The molecular detection of three virulence genes—mecA, femA, and lukE—is examined in this work. 5.7% of multidrug-resistant (MDR) Staphylococcus aureus isolates had their genomic DNA extracted; these bands were verified by gel electrophoresis. A Nanodrop spectrophotometer was employed to ascertain the DNA's purity and concentration. Every DNA-extracted sample has been subjected to PCR. Following gel electrophoresis, the bands are analyzed by comparing their molecular weight to the 100 bp DNA Ladder. A PCR reaction using primer sets of mecA (356 bp), femA (509 bp), and lukE (906 bp) was performed on each DNA-extracted sample. The findings revealed that all isolates of multidrug-resistant S. aureus possessed the FemA and lukE genes, while only 3 possessed the mecA gene, out of 5.7% of MDR S. aureus, as shown in Figure 3.

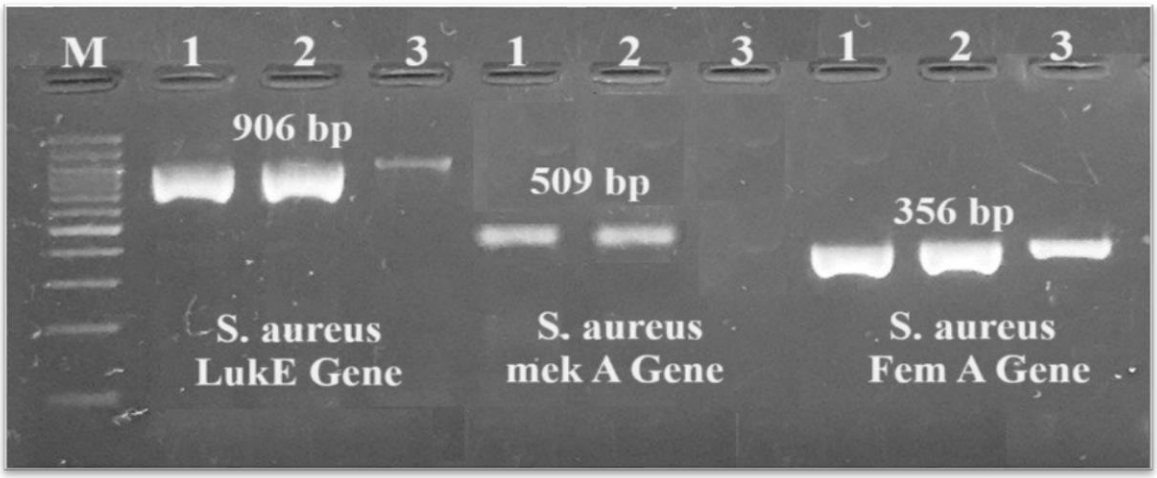

Figure 3. Represents 2% agarose gel electrophoresis of the PCR products showing lane mecA (356 bp), lane femA(509 bp) and lane lukE(906bp).

The role of Staphylococcus aureus in stimulating genes linked to pharyngitis in various immunological cells

The pharyngitis gene expression results, which were analyzed using Graph Prism software, demonstrate a significant difference between cell culture specimens not treated with killed S. aureus and cell culture specimens that were activated with killed S. aureus using RTqPCR.

gene expression in the lymph nodes

The three genes under investigation exhibited elevated expression in the lymph node. The first tested the secretion of TNF-Alpha release in lymph nodes before and after bacterial infection. Figure 4 illustrates the findings, which indicate that TNF-Alpha gene expression rose to 975.5 times following the activation of the lymph nodes with killed Staph aureus cells.

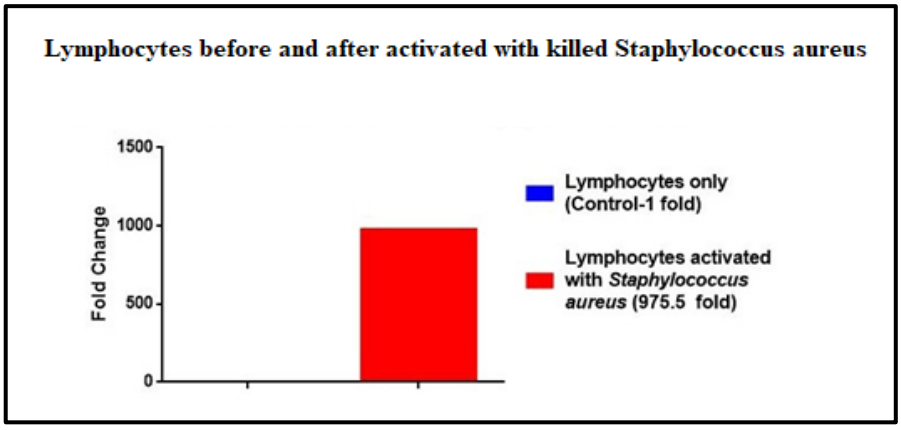

Figure 4. TNF-Alpha gene expression in the lymph nodes

Activation of the lymph nodes by killed Staph aureus cells resulted in a 166.5-fold increase in IL-6 gene expression in the lymph nodes, as seen in Figure 5.

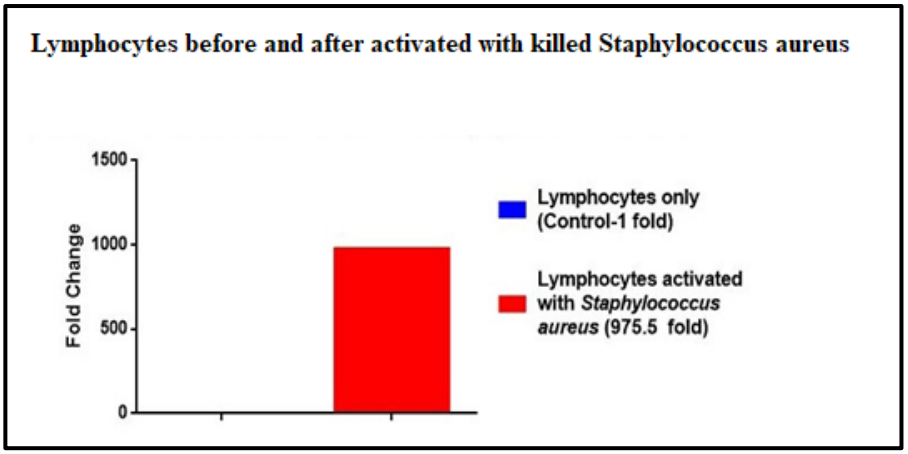

Figure 5.IL-6 gene expression in the lymph nodes

As illustrated in Figure 6, the results indicate that the expression of the NF KB gene in lymph nodes increases by 26.3 times following the activation of the lymph nodes with killed S. aureus cells.

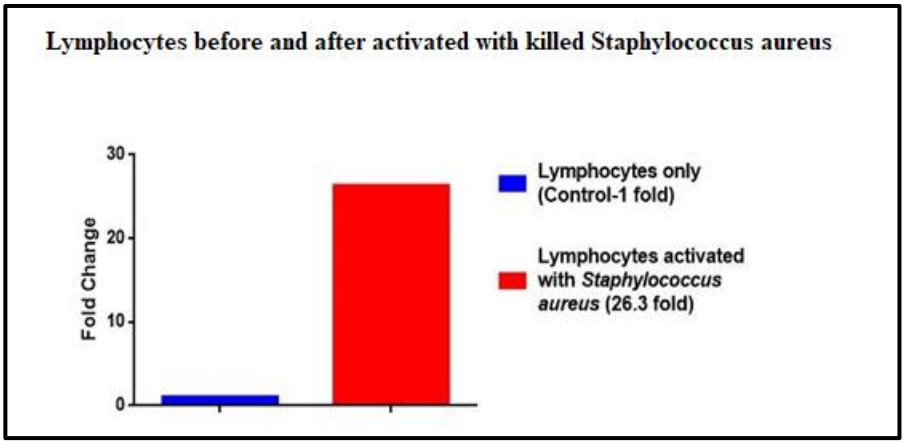

Figure 6. NF Kappa B gene expression in the lymph nodesgene expression in the splenocytes

The same was applied to splenocytes; the findings demonstrated that the TNF-Alpha and IL-6 genes under investigation exhibited elevated expression following activation with killed S. aureus cells. As seen in the image 7, the activation of splenocytes with killed S. aureus cells resulted in a 6.8-fold increase in IL-6 gene expression.

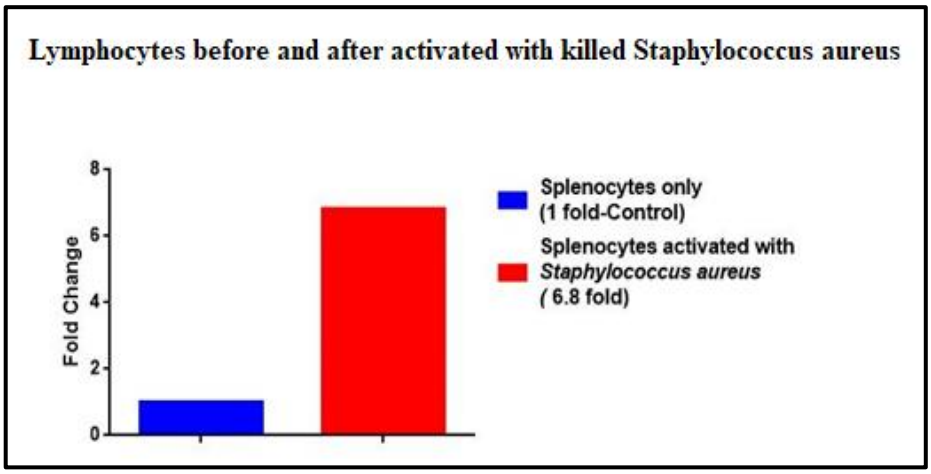

Figure7. IL-6 gene expression in the Splenocytes

On the other hand, the expression analysis of TNF-α genes revealed an increase of 1351.1-fold after activation of the splenocytes with killed S. aureus cells, as seen in Figure 8.

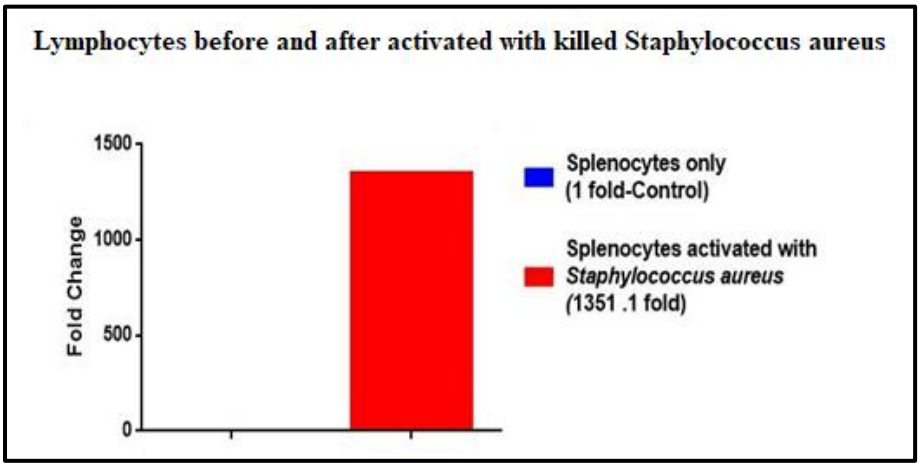

Figure 8. TNF-Alpha gene expression in the splynocytes

DISCUSSION

Bacterial side

The most frequent cause of pharyngitis is Staphylococcus aureus. Its antibacterial resistance and endurance in the tonsil's internal tissues account for its significance. S. aureus was isolated in 57.3% of cases in this study. The bacterial persistence in the tonsils during an inflammatory process and antibiotic treatment is shown by the significant incidence of S. aureus. The areas of the throat and anterior nostrils are thought to be the main places where S. aureus colonies [11] . Children under the age of ten made up 52.8% of the most often infected age group, because of their varied interactions at home, school, and daycare facilities, the oral microbiota is becoming more varied, which raises the incidence of these illnesses [24] . The gender distribution of children with pharyngitis showed that 45.7% were female, and 54.2% were male. This indicates that the prevalence of pharyngitis differed between males and females. These results correspond with Balla et al. [25] , who discovered that more children with pharyngitis infection were females than males. This distribution of genders highlights several possible causes for this discrepancy, including differences in immune responses or sex-specific hormonal profiles, which may impact immune system functioning and make males or females more susceptible to certain infections; genetic differences in immune-related genes that may make males less effective in responding; changes in the respiratory system's structure, as males and females differ slightly; and environmental exposure, as males are more susceptible to environmental conditions, particularly in adulthood, which increases the risk of

infection[26]. Our investigation revealed a 100% resistance rate to oxacillin. This result is consistent with what Kobayashi et al. found [26] .

Cavalcanti claims that the expression of the mecA gene mediates the resistance to oxacillin [11]. Followed by Amoxicillin (91%) and Ceftriaxone (80%). As a moderate resistance was detected for penicillin G. The fundamental explanation for this high prevalence of resistance to penicillins rests on the generated βlactamase enzymes that break the β-lactam ring and inactivate penicillin antibiotics [27] . We discovered that 85.7% of Staph. aureus were resistant to Vancomycin, but Gurung et al. [28] showed 0% of Staph. aureus was the same. Decreased permeability caused by thickening the cell membrane can result in vancomycin resistance by preventing or limiting vancomycin's access to target cell structures [29] . Whereas the resistance rate of Gentamicin was 31%. These results correlated with the results obtained by [30] and [31] , who found the resistance to Gentamicin was 20% and 25%, respectively. The resistance to Gentamicin may be because S. aureus has many ways for aminoglycoside resistance, and most of the genes responsible for this resistance are located on plasmids, which can transfer this resistance between bacterial species [4] . In contrast, the resistance to levofloxacin was 7.1%, 30%, and 32% to azithromycin, chloramphenicol, and levofloxacin, respectively. According to the current investigation, S. aureus had a low level of clindamycin resistance (5.7%). This finding is consistent with that of Ali [30] , who demonstrated that 4% of people had clindamycin resistance. This implies that clindamycin is a superior choice for the treatment of S. aureus-induced pharyngitis. Because clindamycin has higher tissue penetration, fewer side effects, and is less expensive, it has been used to treat severe staphylococcal infections like MRSA [32] .

Molecular side:

The genes responsible for S. aureus's antibiotic resistance are found on chromosomes or plasmids, and there is a chance that genes will spread among strains of the same species [33] . This study was designed to detect the presence of lukE, femA, and mecA genes. According to the results, only three S. aureus isolates out of 5.7% of MDR had the mecA gene. This indicates that the patients have methicillin-resistant S. aureus, which is consistent with Jafar's findings[34] . The key to identifying MRSA is locating the mecA. The MecA gene, which is found in the SCCmec resistance island [35] , encodes for a modified protein (PBP2a) that has a low affinity for β-lactam antibiotics, making the phenotype resistant to these drugs. This increases the pathogenicity of S. aureus at different stages of infection by preventing the lethal effect of antibiotics, as well as the severity of the disease and the bacteria's capacity to colonize and spread in the host tissues [30] . However, not all isolates resistant to oxacillin in our study had the mecA gene, which was demonstrated by a prior study in Nigeria regarding the total lack of the mecA gene and five major types of SCCmec in MRSA. This suggests that the resistance may be caused by an excess of β-lactamase [36] . According to recent research, S. aureus isolates that lack the mecA gene can instead have a variety of cassette genes encoded by other genes, including the mecB, mecD, and mecC genes [37.]

The findings also demonstrated that the femA gene was present in every S. aureus strain. This finding is consistent with that of Kobayashi et al., who demonstrated that all S. aureus isolates carried the femA gene [18] . Since their expression is necessary for encoding proteins that affect S. aureus's level of methicillin resistance, the femA genes play a regulatory role [17] . Compared to other fem genes, femA exhibits a stronger connection with methicillin resistance [18] . In addition, a different biochemical investigation revealed that the femA product might be involved in cell wall production metabolism [38] . Our study's findings also demonstrated that all isolates had the lukE gene. Leukocidins are essential for undermining host-immune defenses [39] , and they also contribute to disease by focusing on primary human leukocytes that are essential for both innate and adaptive immunity [40] . Accordingly, the presence of virulence genes in S. aureus, such as the PVL or mecA genes, enhances the bacteria's virulence and resistance to antibiotics by producing modified gene products [9] .

Immunity side:

Numerous immune system cell types, such as macrophages, lymphocytes, and polymorphonuclear leukocytes, are stimulated by S. aureus to produce and release pro-inflammatory cytokines (TNF-alpha, IL-1β, etc.) and inflammatory mediators, which are crucial in mediating inflammatory responses. It is recognized that cytokines are in charge of the development and spread of inflammation. According to Al-Qahtani et al. [41] , IL-6, IL-1β, and TNF-α are specifically regarded as the most significant pro-inflammatory cytokines. Therefore, in our work, we measured the gene expression levels for these cytokines in splenocytes and lymph nodes. The results demonstrated that following activation with dead Staphylococcus aureus, these tissues' levels of IL-6, TNF-alpha, and NFKB significantly increased. The sera of mice with S. aureus-induced pharyngitis showed elevated levels of TNF-α, according to a study by Jia et al. [42] .

The elevated production of pro-inflammatory cytokines in respiratory infections is consistent with the elevated levels of TNF-α observed in our study and others. Therefore, the inflammatory condition is exacerbated in the respiratory tract when monocytes/macrophages, activated mast cells, dendritic cells, natural killer cells, and lymphocytes release TNF [43] . Prior research has demonstrated that the onset and progression of chronic inflammatory disorders involve the engagement and overexpression of TNF-alpha, IL-6, and IL-1β [44] . Additionally, they are in line with the outcomes of chronic pharyngitis observed in rats in ammonia-induced models [45] . Our findings showed a strong correlation between TNF-α and IL-6 in patients with pharyngitis. TNF-α, a pyrogen cytokine generated from immune cells in response to autoimmune and chronic inflammatory illnesses, also increases the production of IL-6, indicating that IL-6 is a downstream effector of TNF-α [46] . qRT-PCR was also used to assess nuclear factor-κB (NF-κB). The findings demonstrated that activation with dead Staph. aureus boosted NF-κB gene expression in the lymph nodes. Several triggers, such as chemicals originating from pathogens, intercellular inflammatory cytokines, and many enzymes, can quickly and temporarily stimulate the nuclear transcription factor NF-κB [47] . Furthermore, NF-κB is essential for controlling several genes that produce inflammatory mediators [42] . The canonical pathway and the non-canonical pathway are the two distinct pathways by which the NF-κB signal is transduced. The non-canonical pathway stimulates secondary lymphoid organogenesis and immune cell maturation and differentiation, while the canonical pathway stimulates inflammatory responses. This results in controlling the recruitment of inflammatory cells and the production of pro-inflammatory cytokines, both of which fuel the inflammatory response [48] . However, a number of inflammatory illnesses, including cancer, septic shock, and rheumatoid arthritis, will result if the activation of the NF-κB signaling pathway is not stopped in a timely manner [49] .

CONCLUSION

This study demonstrated that the pathophysiology of bacterial pharyngitis depends on the bacteria's induction of the inflammatory host response. Cytokines such as interleukin-6 and TNF-alpha are the most important elements of this reaction. In comparison to the control group, activated cells containing dead Staphylococcus aureus cells showed considerably higher expression of TNF-alpha, IL-6, and nuclear factor-κB (NF-κB) in the lymph node. The gains were greater than 166.5 times, 975.5 times, and 26.3 times, respectively. In contrast, the Splenocytes exhibited 6.8 and 1351.1-fold gene expression for TNF-alpha and IL-6, respectively. The presence of virulence genes identified in this study has also increased the pathogenicity of the bacteria, making them resistant to all used antibiotics.

DECLARATIONS

Author contributions

Conceived and designed the experiments: Karkaz Mohammed Thalij, Marwa Hassan Abdel Wahab.

Performed the experiments: Shahad Muaad Tawfeeq.

Analyzed the data: Shahad Muaad Tawfeeq

Contributed reagents/materials/analysis tools: Shahad Muaad Tawfeeq

Wrote the paper: Shahad Muaad Tawfeeq

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