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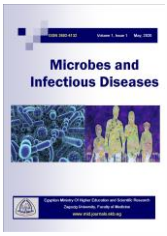

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Original article

Phylogenetic analysis of *Staphylococcus sp.* related with thyroiditis in Al-Nasiriyah City, Iraq

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ABSTRACT

Background: *Staphylococcus* is a Gram-positive (GM^+) facultative anaerobic, non-motile coccus. It's frequently results in a variety of illnesses in human. *Staphylococcus* sp. considered the most common organisms that can infect the thyroid gland. **Methods:** Genomic DNA was extracted from 20 thyroiditis patients' blood samples and analyzed for 16S rRNA using PCR, employing the Universal gene. Gene amplification was performed in a thermocycler under specific cycling conditions, including initial denaturation, annealing, extension, and final extension. For the purpose of partially DNA sequencing for the 16S rRNA gene, six PCR results were selected in order to investigate any possible association between the results and the global records in GenBank. The data was analyzed using Statistical Package for Social Sciences (SPSS) version 27, using chi-square test and basic ratios, with P-values between 0.05 and 0.01 indicating statistical significance. **Results:** Findings showed that 6/20 (30%) were *Staphylococcus* sp. isolates. The 16S rRNA PCR product was registered in GenBank in accordance with the official accession numbers of (PP396084, PP396083, PP396085, PP396086, PP396082 and PP396093). The local *Staphylococcus* sp. isolates have distinct molecular links with those of similar strains worldwide according to the phylogenetic tree generated by the MEGA-10 program. **Conclusions:** Thyroiditis continues to be one the most threating health problems around the world. Thyroiditis patients are more susceptible to GM^+ microorganisms. *Staphylococcus* sp. is an important opportunistic pathogen and it's associated with substantial morbidity rate.

Introduction

Thyroiditis, thyroid gland inflammation encompasses a variety of statuses that may disrupt the gland's normal functioning. This condition is categorized based on the nature of clinical manifestations (whether symptomatic or asymptomatic), the temporal progression of the disorder (acute, sub-acute, or chronic), and the etiological factors involved (such as autoimmune processes, infectious agents, pharmacological interventions, or exposure to radiation) [1].

The term thyroiditis describes a broad range of inflammatory conditions. One rare type of thyroiditis caused by a microbial infection is called acute suppurative thyroiditis [2]. The most prevalent organisms that can infect the thyroid gland are *Staphylococcus* sp. and *Streptococcus* sp. [3,4].

The thyroid gland is susceptible to both acute and chronic inflammatory conditions, which

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may be categorized as either suppurative, characterized by the presence of purulent material, or non-suppurative. Additionally, the thyroid may exhibit pathological changes secondary to systemic infiltrative disorders. Infectious Thyroiditis, an infrequent pathology, is predominantly attributed to bacterial etiology. Pertinent laboratory findings during the acute phase often include a suppressed secretion of thyroid-stimulating hormone (TSH) and concomitant elevations in the serum levels of triiodothyronine (T3) and thyroxine (T4) [5].

One of the most advanced development techniques in molecular biology today is the sequencing process. This allows for the quick detection of genetic relationships and mutations between bacterial isolates [6-8].

This study's objective was to investigate the proposed part that bacterial infections play among thyroiditis patients in Thi-Qar Governorate, Southern Iraq.

Patients and methods Study type and population

This study's objective was to investigate the potential involvement of bacterial infection in Iraqi patients suffering from thyroiditis. Falls within August to November 2023 at Al-Haboby Teaching Hospital and some private clinics in Thi-Qar Governorate. A 20 blood samples were obtained and analyzed using the conventional Polymerase Chain Reaction (PCR) technique, employing the Universal gene for assessment.

Sample collection

A total of 25 blood samples obtained from 20 Iraqi thyroiditis patients were chosen through direct patient interviews, taking into account the findings of thyroid function tests, the diagnosis made by the physicians, and the patients' medical history, and five samples were taken from apparently healthy person, whose gland tests were ideal, who were considered controls. Control samples underwent the same tests in order to compare the outcomes with those of the patients.

Molecular Detection of *Staphylococcus* sp.

Genomic DNA was isolated from the blood samples of thyroiditis patients using a DNA Extraction kit (Favorgen/Austria). Each bacterial isolate was then analyzed for the presence of 16S rRNA (universal gene) through the conventional PCR method, employing specific primer pairs designated for each gene according to table (1). The gene amplification process was conducted in a thermocycler (Hamburg, Germany), with PCR cycling conditions meticulously set according to the primer specifications. The PCR protocol included 1 cycle of initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94 ºC for 1 minute, 1 cycle of annealing at 55ºC for 30 sec, and 35 cycle of extension at 72°C for 1 minute, followed by 1 cycle of final extension of 7 minutes at 72°C as shown in table (2) [9].

Gel electrophoresis:

PCR products for the 16S rRNA gene in *Staphylococcus* sp. isolates were seen at 280 nm under UV illumination following ethidium bromide staining for 30 minutes at 80 v. (Figure 1).

Analysis of the amplified PCR products was done on agarose gel electrophoresis (Agaro-Power™) 2 % stained with ethidium bromide in 1x TBE (Tris-Borate-EDTA) buffer to detect the corresponding amplified fragments. The PCR fragments were all documented by Gel Documentation System and Software for DNA analysis (FSF-SPBT – UK) using 100 bp molecular weight ladder (Fermentas™, Finland) for confirmation of proper PCR product length.

Sequencing Analyses

The 16S rRNA gene of six *Staphylococcus* sp. isolates from thyroiditis patients was partially sequenced, and the PCR products were then compared to reference strains of *Staphylococcus* sp. in the NCBI. The (MEGA-10) program was used to create a phylogenetic tree for the sequenced genes [10].

A partial sequence was performed for all isolates (n = 16), but this study only sheds light on *Staphylococcus* sp. Because they represent the majority of isolates.

For taxonomic identification, phylogenetic analysis, diagnostic applications, epidemiological research, and comparative genomics, the 16S rRNA gene is essential. tracks the spread of certain strains, and serves as a benchmark for comparing the genomes of various strains. It also helps in understanding bacterial diversity, evolution, and pathogenicity is aided by this sequencing.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) program, version 27, was used to analyze the data. Simple statistical ratios like percentage, mean, standard deviation, and chi-square test were used to illustrate the data. Statistical significance was defined as a *P*-value of less than or equal to (0.05) and (0.01).

Ethical considerations

The study obtained ethical permission from Thi-Qar Health Directorate by their agreement coded 174/2023.

Results

From a total of 20 thyroiditis patients blood samples, 16 (80%) samples were given a positive bacterial culture, with a significant predominance, instead of 4 samples (20%) with no growth ($p \leq 0.005$). The diagnosis was done directly using conventional PCR method, which amplifies 16S rRNA. According to Table (2), the outcomes demonstrated that Gram positive bacteria ($GM^{+)}$ were more prevalent than Gram negative bacteria (GM^{-}) with an occurrences of 11 (68.75%) and 5 (31.25%), respectively ($p>0.01$) as displayed in Table (3).

As illustrated in (Figure 2), *Staphylococcus* sp. was the most common isolation among GM+ bacteria with a percentage of 6 (37.5%), then came *Streptococcus* sp. with a percentage of 4 (25%) and *Corynebacterium* sp. with one isolate (6.25%) ($p>0.01$). The current study's findings demonstrated the prevalence of GM^{-} bacteria was lower, with a proportion of 2 (12.5%) for *Klebsiella* sp. and 1 (6.25%) for each of *Salmonella* sp., *Escherichia* sp. and *Mycoplasma* sp. ($p>0.01$).

Detection of 16S rRNA

By amplification of the 16S rRNA gene, all isolates (n=16) were identified as *Staphylococcus* sp. and other bacterial species using a standard PCR approach. The outcomes demonstrated that every isolate tested positive for the targeted gene, with products of approximately 1500 bp.

The process needs to make it possible to identify bacteria quickly and precisely. 16S rRNA gene gel electrophoresis after ethidium bromide staining [11,12].

Phylogenetic analysis

These six chosen *Staphylococcus* sp. strains officially assigned the GenBank accession numbers of (PP396084, PP396083, PP396085, PP396086, PP396082 and PP396093).

The phylogenetic tree revealed that the local isolates of *Staphylococcus* sp. differed from similar ones globally in terms of their molecular linkages as shown in Figure (3).

A phylogenetic tree is comprised of three genetic groups;

First group: included PP396084 was genetically closer to PP292037.1 in India, and PP092040.1 in France. And PP396083 was genetically closer to PP125047.1 in Russia, and PP494171.1 in Egypt.

Second group: included PP396085 and PP396086 which were closely related to each other and clustered with PP125044 isolated in Russia.

Third group: included PP396082 was genetically closer to PP494162.1 isolated in Egypt, and MW595974.1 in India. And PP396093 was genetically closer to MT261812.1 isolated in Korea (Figure 4).

Table 1. Sequence of primers used in the present study.

primer		5'-sequence-3'	Reference
Universal gene (16S rRNA)	27 F	AGAGTTTGATCMTGGCTCAG	[13]
	1492 R	TACGGYTACCTTGTTACGACTT	

Table 2. PCR conditions for universal gene.

Steps	Temperature (°C)	Time (min)	No. of cycle
Initial denaturation	94	5	1
Denaturation		1	35
Annealing	55	0.5	
Extension	72	1	
Final Extension			1

Table 3. Types of bacteria that were isolated.

Type of bacteria	No. (%)	*p*-value
GM^{+}	11 (68.75%)	NS
GM^{-}	5 (31.25%)	NS
Total	16 (100%)	

NS: Non-significant.

Figure 1. 16S rRNA gene electrophoresis on agarose gel. M:3000 bp ladder; Lanes (5, 6, 10, 12, 13, 14, and 15) showed positive results for *Staphylococcus* sp., with a product size of approximately 1500 bp.

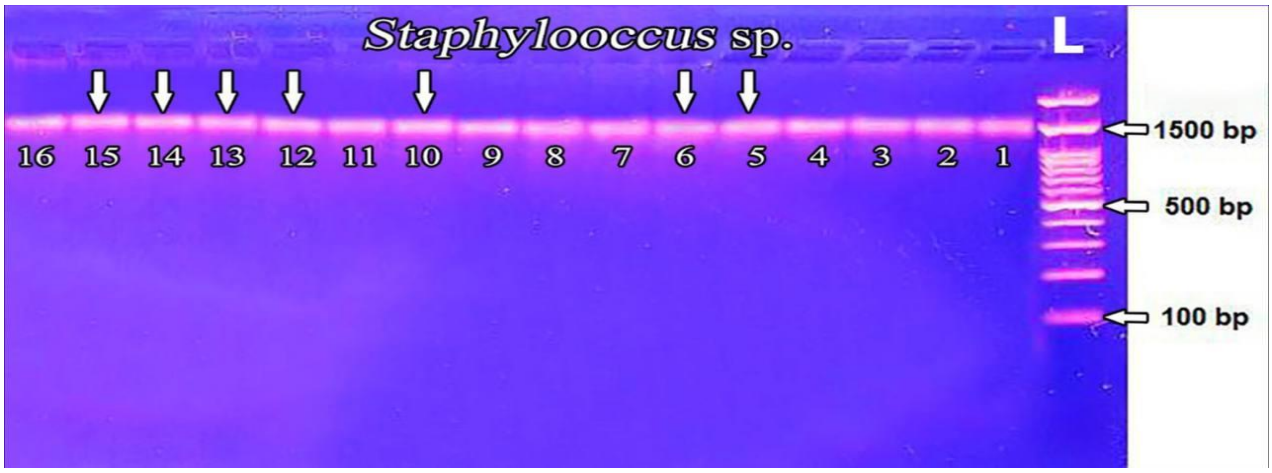


Figure 2. The percentages of GM+ and GM- bacteria that were directly extracted from blood using PCR.

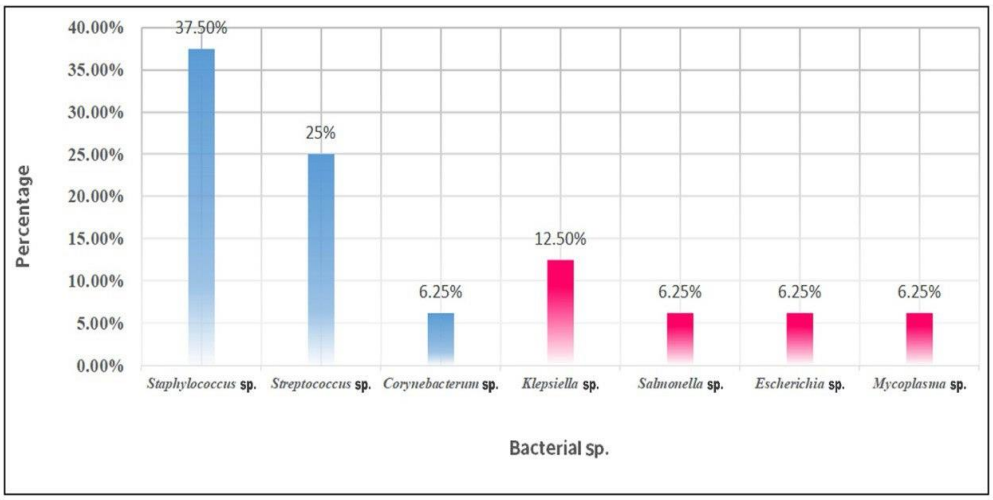
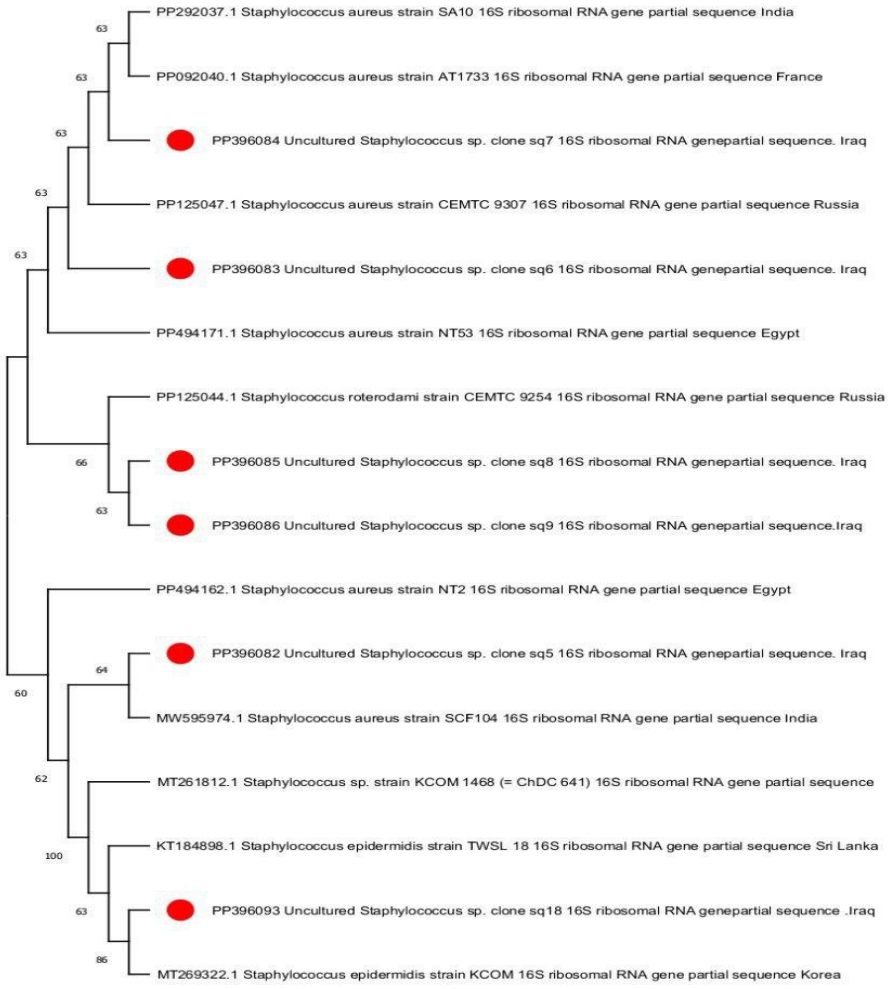


Figure 3. The neighbor joining phylogenetic tree study is based on the gene relationship analysis of (16s RNA) of local isolates of *Staphylococcus* sp. and similar strains.



Discussion

In the context of endocrinological pathology, a diverse spectrum of disorders may precipitate inflammatory responses within the thyroid gland. Primary infections of the thyroid are considered an uncommon clinical occurrence, attributable to an array of microbial entities, with bacterial organisms representing the predominant category. Less frequently implicated pathogens encompass fungal species, parasitic organisms, and viral agents. Among bacterial pathogens, GM^+ cocci, notably staphylococcal strains, are frequently identified as the etiological factors in both adult and pediatric. Furthermore, in individuals with compromised immune function, a variety of opportunistic microorganisms have been documented [14]. In adults, this case arises from the dissemination of microbial organisms through hematogenous or lymphatic channels [15-20]. Thyroid infection in children may result from congenital abnormalities [21]. Other less frequent, etiological factors include direct physical trauma such trauma may occur during medical procedures such as fine needle aspiration biopsies, accidental ingestion of foreign objects, like fish bones [15]. As well as septic emboli caused by infectious endocarditis [22].

The present study findings agreed with a study conducted in Al-Ramadi, Iraq on women suffering from thyroiditis revealed the presence of many bacterial isolates related to both GM^+ and GM^- cultures, the predominant bacteria was *Staph. lentus* [23]. The results of the study are consistent with two global studies, the first was conducted in Sweden that showed the majority of cases of acute

suppurative thyroiditis are caused by bacterial pathogens included *Staph. aureus, Strep. pyogenes, Staph. epidermidis*, and *Strep. pneumoniae.* Seldom additional causative bacteria included *Klebsiella* sp., *Salmonella* sp., *Hemophilus influenzae, Strep. viridans, Arcanobacterium haemolyticum, Eikenella corrodens* and Enterobacteriaceae [24]. While the second study recoded *Klebsiella, Salmonella, Acinetobacter, Pseudomonas, Brucella* sp., *Pasturella* sp., as well as *E. coli* are less common causes of suppurative thyroiditis [17].

In summary, our study contributes to the understanding of thyroid infections by providing insights into the etiological factors, microbial profiles, and clinical implications. However, we acknowledge limitations such as our small sample size and geographic specificity. Due to time constraints, patient non-compliance, and the high cost of PCR technology, the sample size obtained was small. Future research should explore additional aspects of thyroid infections, including long-term effects and specific risk factors.

Conclusion

Thyroiditis continue to be one the most threating health problems around the world. Thyroiditis patients are more susceptible to GM^+ microorganisms. *Staphylococcus* sp. is an important opportunistic pathogen and it's associated with substantial morbidity rate.

More microbial survey studies may be needed to detect other associated microbial infections in morbidity in Thyroiditis patients.

Educational programs for Thyroiditis patients seems to important which focusing on possible risk factors related to disease distribution.

Author contributions

All authors had seen and approved the submission of the manuscript with full responsibility and this research had not been published or under consideration by any other journal.

Conflict of interest

No conflict of interest related to the work was declared.

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