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# An Identification of *Staphylococcus* spp. by PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) Analysis of *dnaJ* Gene and *ApoI* Enzyme

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## Abstract

Genetic methods based on Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) are widely used for microbial species determination, in this study, PCR-RFLP technique was used to target the intragenic between 16S and 23S rDNA (*dnaJ* gene) for rapid detection and identification of twenty *Staphylococcus* species isolates. The RFLP analysis of the *dnaJ* gene and the digestive enzyme *ApoI* was designed for a rapid and accurate identification of the selected genome. In this assay, the conserved intragenic region between 16S and 23S fragments subsequently digestion of the amplicon with restriction enzymes. Therefore, *dnaJ* gene with digestive enzyme *ApoI* were studied to differentiate Staphylococcus genus. The results confirmed that *S. epidermidis, S. hominis,* and *S. lugdunensis* had 3 bands and *S. aureus* 4 bands. However, the restriction enzyme not fail but there are no restrict site in there amplified genome segment for *S. haemolyticus, S. xylosus* and *S. auricularis*. Our study found that a restriction enzyme *ApoI* does not benefit in classifying the species of the genus *Staphylococcus* spp.

**Keywords**: *Staphylococcus* spp., *DnaJ* gene, *ApoI*, PCR-RFLP.

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# تشخيص الأنواع التابعة للمكورات العنقودية بوساطة تقنية PCR-Restriction Fragment Length Polymorphism Analysis باستخدام الجين *dnaJ* والانزيم القاطع *ApoI*

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## الخلاصة

تم دراسة الجين *dnaJ* مع الانزيم القاطع *ApoI* في تفريق الانواع التابعة لجنس *Staphylococcus* spp. بتقنية PCR-RFLP وجد ان الانواع *Staph. epidermidis* و *Staph. hominis* و *Staph. lugdunensis* يمتلكون 3 حزم، بينما *Staph. aureus* 4 حزم، اما بقية الانواع المتمثلة بـ *Staph. auricularis* و *Staph. haemolyticus* و *Staph. xylosus* فقد فشل الانزيم القاطع *ApoI* بفصلهم الى حزم، تبين من دراستنا ان الانزيم القاطع لا يفيد في تصنيف الأنواع التابعة لجنس المكورات العنقودية

**الكلمات المفتاحية:** PCR-RFLP، الانزيم القاطع *ApoI*، الجين *dnaJ* .

## Introduction

*Staphylococcus* species are found in habitats and isolated form human and animal [1]. Different methods were used for distinguish *Staphylococcus* spp. based on phenotypic and genotypic features. Unfortunately, the accuracy of these frameworks are ranged from 50 to 70, a specific recognition region known as restriction sites are cleaves DNA into small pieces at or near [2]. Restriction Fragment Length Polymorphism (RFLP) technique is used to digest the PCR product by restriction enzyme like *ApoI* to obtain several bands belong to the selected genus [4]. Moreover, conventional reference methods are too laborious and time consuming to be used in clinical laboratories. Several problems that associated with the systems mentioned above result from the variability in the expression of metabolic activities and / or the morphological features of some staphylococcal species [3]. The traditional methods do not give the correct

results of the classification of the Staphylococcus genus to a level type, so it has been relied on advanced molecular methods for the purpose of more accurate diagnosis; Due to the limited number of stable features that can used for species discrimination, many taxa remain difficult to distinguish from one another and are misidentifies by phenotypic tests [5]. The *dnaJ* gene is responsible for coding DnaJ protein, known as Hsp40 one of the heat-shock proteins of a genus Staphylococcus, which is one of the genes known housekeeping genes to be responsible for classifying *Staphylococcus* spp., and this gene is highly accurate in the diagnosis of species belonging to this genus in terms of phylogenetic analysis [6]

This study describes a benefit of RFLP technique for detection *Staphylococcus* spp. used *dnaJ* gene and distinguish between them using *ApoI* restriction enzyme.

## Materials and Methods

### Bacterial Isolates

All isolated bacteria of *Staphylococcus* strains were obtained from facilities of Dept. of biology / Coll. of science / Uni. of Mosul, each isolate was recognized morphologically, biochemically and molecularly.

We selected the twenty local isolates belongs to *S. aureus, S. auricularis, S. lentus, S. haemolyticus, S. lugdunensis, S. epidermidis, S. hominis, S. cohnii, S. intermidius* and *S. xylosus* and references isolates (ATCC) for comparison between them table 1.

The Deoxyribose Nucleic Acid was extracted by using Wizard® Genomic DNA Extraction Kit following the manufacture instructions (Promega Corporation-USA).

**Table 1:** List of the reference *Staphylococcus* spp. American Type Culture Collection (ATCC)

| | | | |
|---|---|---|---|
| 1 | *S. aureus* 43300 | 6 | *S. hominis* 27844 |
| 2 | *S. auricularis* 33753 | 7 | *S. intermidius* 29664 |
| 3 | *S. cohnii* 20260 | 8 | *S. lentus* 29070 |
| 4 | *S. epidermidis* 14990 | 9 | *S. lugdunensis* 43809 |
| 5 | *S. haemolyticus* 29970 | 10 | *S. xylosus* 29971 |

**Detection of *dnaJ* gene**

The selected primers were obtained from (Alpha DNA Company, Montreal, Quebec-CANADA) and subjected into magnify the *dnaJ* gene piece upstream 5-GCC AAA AGA GAC TAT TAT GA-3 and downstream 5-ATT GYT TAC CYG TTT GTG TAC C-3. The PCR program incubation for 3 minutes at 94°C and following 5 rotations at 94°C for 30 seconds, 45°C for 30 seconds, and 72°C for 60 seconds after that pursued a series of 30 rotations at 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 60 seconds; these were achieved for a ultimate expansion at 72°C for 3 minutes and 4°C for 3 minutes [6].

The PCR reaction output was assured by 1% (wt/vol) agarose gel electrophoresis and imagination via ultra violet illumination [**6**].

**Limitation enzyme *ApoI***

This method includes restriction enzyme *ApoI*. Overhang 5′-AATT (Time-Saver, Bio Labs New England) with some modification this technique achieved by 5µl of the Polymerase Chain Reaction output in an overall size of 15 µl for 1X stimulus buffer for 10 U of the *ApoI* endonuclease for 3 h at 37°C, the resulting fragments is detached through electrophoresis on 2% agarose gel and was imagined via ultra violet illumination [7].

## Results and Discussion

After amplified *dnaJ* gene (references and local), the results displayed that all references isolate (with exception *S. lentus*) have this gene.

The local isolates were belonging to *S. aureus*, *S. auricularis*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis* and *S. xylosus* species contain *dnaJ* gene however *S. cohnii*, *S. intermidius* and *S. lentus* did not show any results figure 1.

**An Identification of *Staphylococcus* spp. by PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) Analysis of *dnaJ* Gene and *ApoI* Enzyme**

**Aws Ibrahim Sulaiman**

Depending on results, not all locally strains contained this gene, may be because our environmental and geographical status or mechanisms of gene transfer and different of restriction enzyme used all of these parameters may be effect on this gene. figure 2.

This result was similar somewhat to Naushad and their colleagues [8] when they study about the relationships among Staphylococcus species using 16S rRNA gene, *dnaJ*, *rpoB*, and *tuf* gene and they demonstrated that *dnaJ* gene size was 816 bp. This method it is very important to identification the *Staphylococcus* spp. [5 and 9].

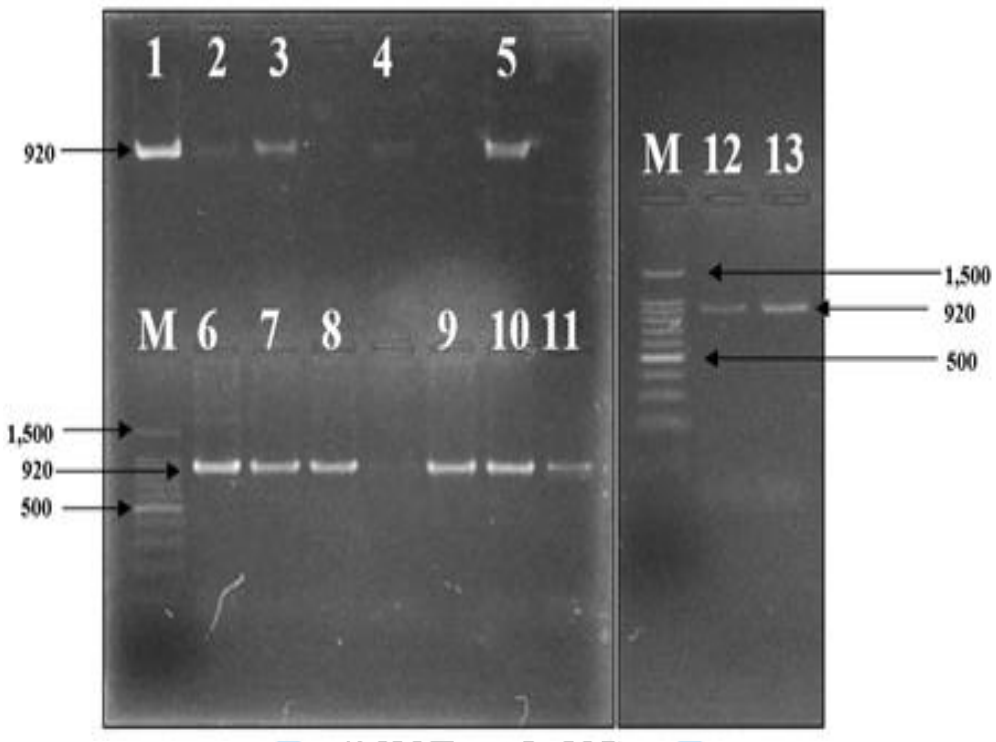


**Figure 1**: dnaJ gene after electrophoresis by agarose (920 bp.), M ladder ,1, 12 S. hominis, 2,5 S. epidermidis, 3,10,11 S. lugdunensis, 4 S, haemolyticus, 6,7,8 S. aureus, 9 S. xylosus and 13 S. auricularis

Diyala Journal for Pure Science

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**Aws Ibrahim Sulaiman**

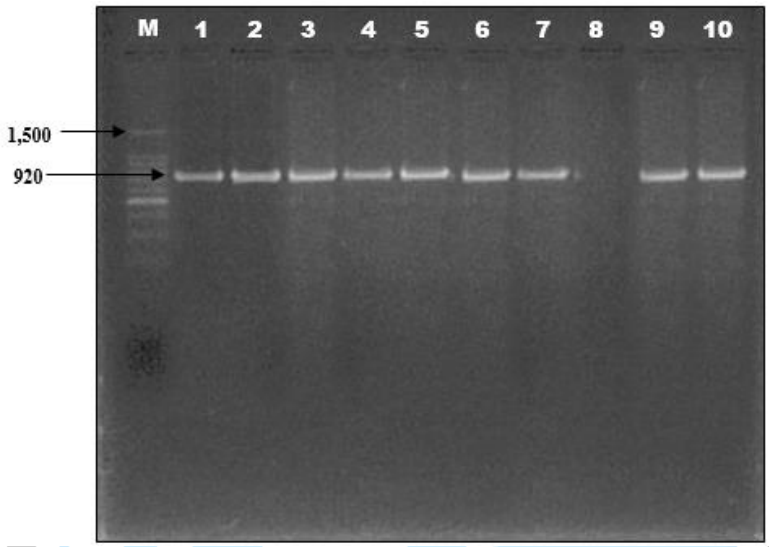


**Figure 2:** dnaJ gene after electrophoresis by agarose (920 bp.), for references Staphylococcus spp., M: ladder 1. S. aureus, 2. S. auricularis, 3. S. cohnii,4. S. epidermidis, 5. S. haemolyticus, 6. S. hominis, 7. S. intermidius, 8. S. lentus, 9. S. lugdunensis and 10 S. xylosus.

After that, we differentiated between species and subspecies by restriction enzyme *ApoI*, figure 3 and figure 4. The results after *ApoI* digestion enzyme showed that *S. epidermidis S. hominis* and *S. lugdunensis* have 3 bands and *S. aureus* 4 bands. However, the digested enzyme failure to separated bands of *S. haemolyticus, S. xylosus, S. auricularis*. When the result was compared with references genome, we found that *S. epidermidis* and S. *lugdunensis* have 3 bands, *S. aureus* and *S. hominis* has 4 bands, the rest genomes *S. haemolyticus, S. xylosus* and *S. auricularis* have 4.

This result was similar to some researcher's study; they identified *Staphylococcus* spp. and found that, the local isolates of *Staph aureus* has 3 bands as a comparison with the reference isolate (2 bands). Also, *Staph. lugdunensis* has 3 bands and reference has 2 bands. Not all restriction enzymes had the ability to produce the same bands between local isolates and reference isolates for bacteria otherwise Polymerase Chain Reaction-Restriction Fragment Length Polymorphism test is very beneficial into the taxonomy of *Staphylococcus* spp. and demonstrates into mild, minimal costly and minimal devices attached than sequencing [10**].**

**An Identification of *Staphylococcus* spp. by PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) Analysis of *dnaJ* Gene and *ApoI* Enzyme**

**Aws Ibrahim Sulaiman**

Hauschild and Stepanovic, [7] found that *dnaJ* was used for identification and phylogenetic analysis not for *Staphylococcus* spp. only but for other bacterial species, and they obtained 4 bands from *S. aureus, S. auricularis, S. haemolyticus* and *S. xylosus*, 3 bands from *S. epidermidis, S. hominis* and *S. lugdunensis*. This result approximately near to our result, the only different that, they used digestive enzyme *XapI*. Moreover, our isolates are locally and may be have another characters different from others, these results are similar to Martineau and their colleagues study, [11] and Khademi and their coworkers [9]. They found that the relationship between *S. epidermidis, S. aureus, S. lugdunensis* are very closely, whereas *S. epidermidis* has subgroups belong to *S. epidermidis, S. hominis* and *S. haemolyticus* [6].

DNA analysis techniques may stock a preferable standby into the consistency about Staphylococci genus in order to their higher specificities and susceptibility, there are some molecular bases have been hard-done by molecular consistency of *Staphylococcus* spp., inclusive the 16S rRNA, *femA* , *sodA* and *rpoB* genes, all of them are bases have been hard-done by the molecular investigation technology like hybridization and the sequencing [5,12 and 13].

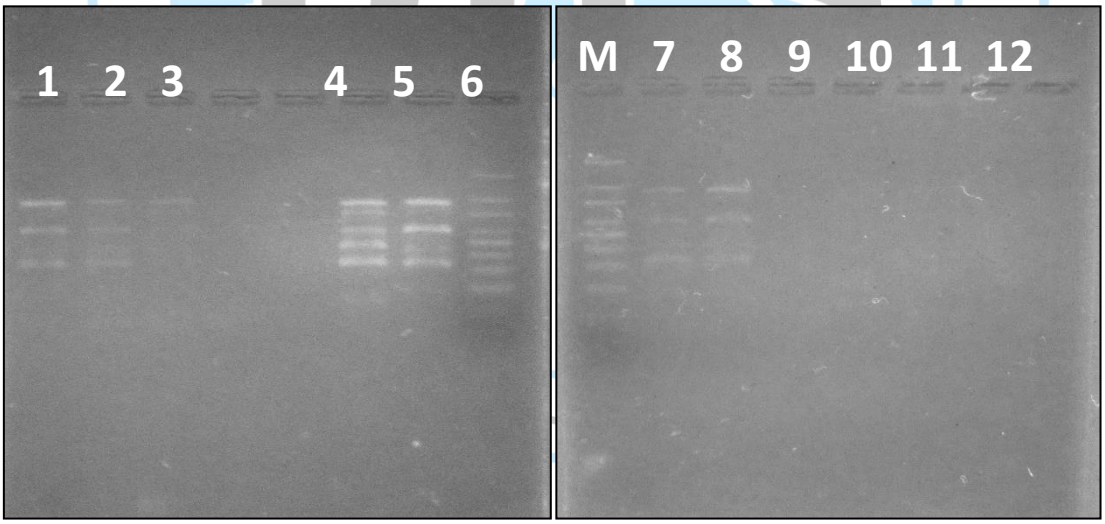


**Figure 3:** Separated bands used *ApoI* assimilation of Polymerase Chain Reaction output, 1, 12 *Staph. hominis*, 2,5 *Staph. epidermidis*, 3,10,11 *Staph. lugdunensis*, 4 *Staph, haemolyticus*, 6,7,8 *Staph aureus*, 9 *Staph. xylosus* and 13 *Staph. auricularis*

Diyala Journal for Pure Science

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**Aws Ibrahim Sulaiman**

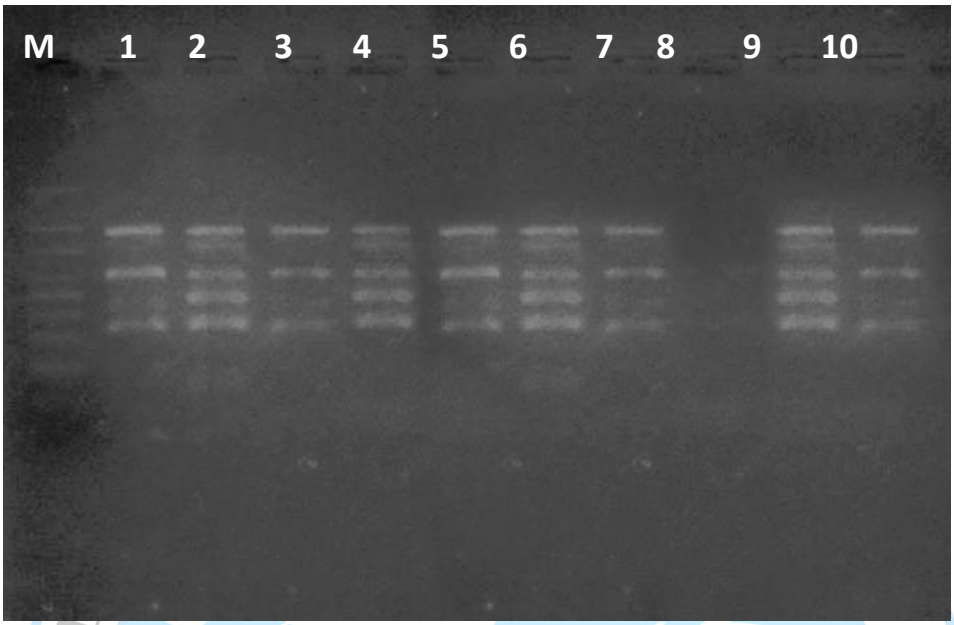


**Figure 4:** Separated bands used *ApoI* assimilation of Polymerase Chain Reaction output, for references *Staphylococcus* spp., M: ladder 1. *Staph. aureus,* 2. *Staph. auricularis*, 3. *Staph. cohnii*,4. *Staph. epidermidis,* 5. *Staph. haemolyticus,* 6. *Staph. hominis*, 7. *Staph. intermidius*, 8. *Staph. lentus,* 9. *Staph. lugdunensis* and 10 *Staph. xylosus.*

## Conclusion

The current research has exhibited that Polymerase Chain Reaction examination of *dnaJ* gene and *ApoI* assimilation is a sufficient device into exact consistency of nearly whole common members of *Staphylococci,* This method is less expensive to compare between *Staphylococcus* spp., Making the most of this research is to separate the species belonging to the genus Staphylococcus in a way that minimizes the error rate so that we get separate strains very accurately.

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