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Iran. J. Ichthyol. (ICAEAS issue 2021): 16-20
P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

## Research Article

# Molecular detection of *spa* gene among *Staphylococcus aureus* isolated from mastitis

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**Abstract:** From June to September 2020, among thirty gram-positive bacteria grown with mannitol salt agar, seventeen *Staphylococcus aureus* isolates were described. *Staphylococcus aureus* isolates were described in the initial identification based on the colonial morphology, microscopic analysis, and biochemical tests. The final identification was performed using an automated VITEK-2 compact system. The *spa* genes were detected by the genotyping method using the polymerase chain reaction (PCR) technique. The results showed that the *spa* gene comprised 19 isolates (63.3%) of *S. aureus*.

**Keywords:** Firmicutes, PCR, *Spa* gene, Mastitis.

**Citation:** Ghani Al-Muhanna, S.; Ameer Al-Kraety, I.A. & R. Banoon, S. 2021. Molecular detection of spa gene among *Staphylococcus aureus* Rosenbach, 1884 isolated from mastitis. Iranian Journal of Ichthyology 8(ICAEAS 2021): 16-20.

## Introduction

The main pathogen associated with various clinical forms of mastitis is *Staphylococcus aureus* (Aires-de-Sousa et al. 2007). Among the different forms of induced mastitis by *S. aureus*, subclinical cases have particular significance, because they are unnoticed and largely affect livestock production (Sulieman et al. 2012). *Staphylococcus aureus* plays an important role in clinical and subclinical mastitis considering as one of the most well-known etiologic agents characterizing persistent and recurring, low-cure infections in antimicrobial treatment (Gao et al. 2017). *Staphylococcus aureus* has many virulence factors that include the surface *IgG* binding protein A (*spa*) that its characteristic and function is to capture the Fc region of immunoglobulin of most mammalian species; therefore, prevent phagocytosis of the bacterial cells with the host immune system (Foster 2005).

The gene harboring and encoding protein A (*spa*) consists of some clear and distinct functions: Fc binding, X-region and a C-terminus region, a sequence for cell wall attachment. The X-region of the *spa* gene contains 24-bp repeats with a different number (Kuzma et al. 2005), this allows the study of the genetic diversity in *Staphylococcus aureus* strains as a molecular marker for epidemiological research of source of infects and the comparison of differences in virulent phenotypes between the strains (Choudhary et al. 2018). During recent decades, *S. aureus* has employed or developed a wide range of phenotyping and genotyping processes. RAPD typing and amplification rRNA 16S-23S have also been used as a method for understanding of pathogenic origins and mechanisms of transmission (Cremonesi et al. 2013; Banoon et al. 2019). Thus, this study aimed to detect *spa* gene among the strains of *S. aureus* isolated from mastitis.

## Materials and Methods

**Specimen's collection and bacterial identification:** Thirty specimens of cattle with clinical mastitis were

**Table 1.** PCR program of *spa* primer that apply in the thermocycler.

| Gene | Initial denaturation | No. of cycles | Denaturation | Annealing | Extension | Final extension |
|---|---|---|---|---|---|---|
| *Spa* | 94˚C for 5min | 34 | 94˚C for 60sec | 55˚C for 60sec | 70˚C for 30sec | 72˚C for 5min |

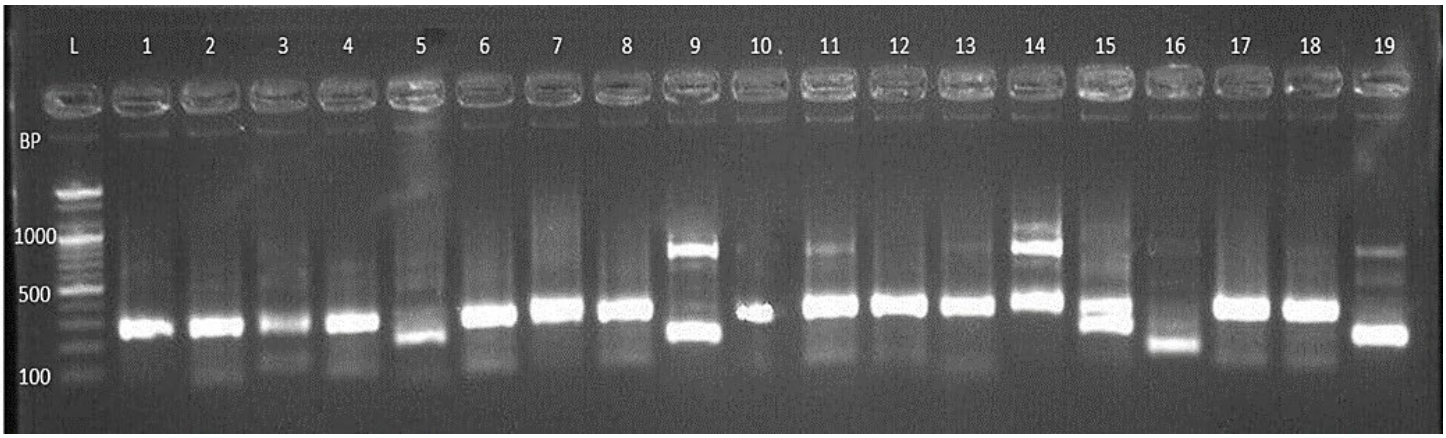


**Fig.1.** *Spa* amplicon (X-region) of *Staphylococcus aureus* isolates on agarose gel electrophoresis.

collected. The samples were inoculated onto nutrient broth, then swabbed onto nutrient agar, then incubated at 37ºC overnight. Bacterial colonies were observed for morphology, size, and consistency. Gram stain was used to determine the gram-negative organism strains and the isolates were streaked onto mannitol salt agar then incubated in aerobic conditions at 37°C for 24 hours, then a single pure isolated colony was transferred to trypticase soy agar for preservation and to carry out other biochemical tests and VITEK system that confirmed the identification of isolates according to (BioMerieux 2010).

**DNA Extraction:** Genomic DNA was extracted by using a commercially extraction kit (Geneaid Biotech Ltd /Taiwan).

**Molecular Identification:** UV transilluminator gel electrophoresis was used to detect DNA. The *spa* genes for the PCR test were detected for *S. aureus* according to Frenay et al. (1996) using F: 5'CAAG CACCAAAAGAGGAA3' and R: 5'CACCAGGTT TAACGACAT3' primer. This primer was designed by Alpha DNA Company, Canada. For the detection of this gene; The Chromosomal DNA extracted from all isolates were subjected to primers by monoplex PCR. The mixture of PCR with final volume 20µl/reaction and the protocol used depending on Master Mix (AccuPower® PCR PreMix (Bioneer, Korea) instructions. Each monoplex PCR reaction mixture consisted of 2µl Forward Primer (10 picomole), 2µl Reverse Primer (10 picomole), 9µl De-ionized water, and 7µl the DNA of the isolates were added into the AccuPower® Taq PCR PreMix tubes that contain (Taq DNA polymerase, dNTPs, KCl, MgCl2, and buffer). All PCR components were assembled in the PCR tube. The PCR reactions Conditions for other steps for each primer as described in Table 1. In order to determine the PCR product size, amplified products have been verified using 0.9% agarose gel electrophoresis. The gel was stained with 4 µL (BioBasic, Canada) 10mg/mL of ethidium bromide and runs 1.5h at 70v. On a UV light transmitter (Cleaver, UK) the bands were observed; images used a gel documentation system (Cleaver, UK). The molecular weights of amplified products were calculated by a 100 bp ladder (Bioneer, Korea) (Levy et al. 2008).

## Results and Discussion

**Identification of bacteria:** Some criteria including

culture, morphology and biochemistry tests are used to determine the first identification of bacterial specimens. The identification of the Gram-negative bacterium was confirmed by the vitek-2 system using a kit (GP-ID cards). The confidence level ranges from very good to excellent with the ID message, probability percentage from 95 to 99.

**Gram positive cocci:** *Staphylococcus* spp. grow on mannitol salt agar and give catalase positive result (Chelikani et al. 2004). Only *S. aureus* gave coagulase positive result, which is a specific test for differentiation of *S. aureus* from coagulase negative *Staphylococcus* (CONS) (Tiwari et al. 2008). The high salt concentration inhibits the growth of most bacteria other than *Staphylococcus*. On mannitol salt agar, pathogenic *S. aureus* produces small colonies and color of medium is changed from pink to yellow due to fermentation of the mannitol sugar and producing acid which, in turn, changes the indicator from pink to yellow (Bachoon et al. 2008). The final identification of bacterial isolates was carried out using automated vitek-2 compact system and the result revealed that from 30 isolates 15 (53.3%) isolates were identified as *S. aureus*.

**Genotyping detection of *Spa* gene in *S. aureus*:** The results revealed that 19 (63.3%) of *S. aureus* isolates possess *spa* gene. These results were in agreement with Kalorey et al. (2007), Klein et al. (2012) and Stephan et al. (2001) who identified *spa* (X-region) gene in *S. aureus* isolates with incidences of 76.5, 70.3 and 85.9%, respectively and disagree with the findings of other studies (Dalla Pozza et al. 1999; Kumar et al. 2010; Coelho et al. 2011; Memon et al., 2013; Kahl et al. 2016; Abdel-Tawab et al. 2016) that established the presence of *spa* X-region gene in nearly all of the isolates. *Spa* (X-region) gene considered as one of the most often and important used methods primarily based on single locus sequencing (Mitra et al. 2013). It is a very common and popular technique used for genotyping staphylococci from mastitis (Lundberg et al. 2016). The *spa* genes are considered to be the most common method or an extra typing system to monitor staphylococcal mastitis for *S. aureus* isolates. The *spa* gene coded protein A is one of the factors of virulence involved in the pathogenesis of *staphylococcus.* Amplification of variable size depending on the number of tandem 24bp are formed by amplifying the X-region of the *spa* gene and this organism is used by scientists to distinguish between different isolates (Khichar et al. 2014). In the X-region of the *spa* gene, the number of repeats is related to the strain virulence. In this analysis, all animal isolates developed five-type *spa* amplicons ranging from 200 to 900 bp. The X-region *spa* gene is produced with cattle tock isolates. Five different types of *spa* amplicons were developed by cattle isolates. 200, 280, 300, 380, 900 bp of 1, 4, 6, 8 and 3 repeats as shown in Figure 1. Marques et al. (2013) reported *spa* gene has been found in all bovine mastitis isolates with the prevalent scale of the variable amplicon of 300 bp. Uniform amplicons of 300 bp, obtained by Sulieman et al. (2012) in 20 isolates of *S. aureus* subclinical bovine mastitis, were not compared to the findings of the present results. Shakeri et al. (2010) also reported that *S. aureus* isolates are spa-deficient.

## Conclusion

The variations in *spa* genes among clinical isolates were significantly higher. The X-region of the *spa* gene can be used in the study of genetic diversity in the *Staphylococcus aureus* strains as a molecular marker for epidemiological research of origin and origins of infection.

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