

## IMMUNOLOGICAL AND MOLECULAR IDENTIFICATION OF THE BACTERIA *STAPHYLOCOCCUS AUREUS* FROM COW'S MILK MASTITIS

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**ABSTRACT :** Bacteria *Staphylococcus aureus* was among the causes of cow mastitis. This bacterial infection was widely recognized through a conventional technique such as used of cow's milk samples cultured on mannitol salt agar (MSA). This test, however, has many disadvantages such as low sensitivity. Three hundred and fifty samples of cow's milk have been obtained from Basra Governorate. Used the screening and agglutination test of latex and 23S rRNA based on the PCR gene, the *S. aureus* was isolated using the traditional procedure. As the reference tool, among the two tests analyzed and compared were sensitivity, accuracy, positive predictive value (PPV) and negative predictive value (NPV). MSA, *Staphylococcus* spp. were isolated from all tested milk samples (350) by blood agar media. The current results, revealed that there were 78.6% (275/350) and 81.8% (225/275), the bacteria isolates appeared good outcome for mannitol salt agar and latex agglutination procedure respectively. The difference among these tests were considered to be highly significant ( $\chi^2=60.824$ ;  $df=2$ ;  $p=0$ ). One hundred fifty (54.5) positive 23S rRNA gene-based PCR isolates were also positive for both MSA and latex agglutination. Based on 23S rRNA gene sequence search in Ribosomal Database together with the amino acid and phylogenetic network analysis, *Staphylococcus aureus* strain UOB\_1 was assigned as the type strain of the genus *Staphylococcus*. The sequence was deposited in GenBank with the accession number MT950107. The calculated efficiency of 23S rRNA gene-based PCR and Latex agglutination in order to diagnosis of bacteria *Staphylococcus aureus* from cow samples milk mastitis compared to manitol salts agar media revealed that both tests showed 100% sensitivity, 40% specificity, 66.66% PPV and 100% NPV. In conclusion, 23S rRNA gene-based PCR and Latex agglutination were considered for the identification of bacteria *Staphylococcus aureus* due to its high sensitivity and negative present value, these two tests were significant.

**Key words :** Mastitis, cow, *Staphylococcus aureus* 23SrRNA.

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### INTRODUCTION

*Staphylococcal mastitis* is one of global important causes of economic losses in dairy production (Ismail, 2017; Mbindyo *et al*, 2020). Dairy cows are susceptible to mastitis during early dry period and transition (3 weeks before parturition and 3 weeks after parturition) or periparturient periods with *S. aureus* being reported as a major pathogen (Pinedo *et al*, 2012). Coagulase positive *Staphylococcus aureus* is belong to the most common contagious mastitis pathogens in dairy cows, with an estimated incidence rate of 43–74% (APHIS, 2007; Kerro Dego *et al*, 2020). Beside that coagulase-negative *Staphylococcus* species (CNS) such as *S. chromogenes*, *S. simulans*, *S. xylosus*, *S. haemolyticus*, *S. hyicus* and *S. epidermidis* are increasingly isolated from bovine milk and being the most increasingly diagnosed causative agent of subclinical mastitis (De

Vlieghe *et al*, 2012; Vanderhaeghen *et al*, 2014; Nyman *et al*, 2018). The bacteria will multiplied rapidly after entered the mammary gland through the nipple canal, adapted to the udder environment and initiated an inflammatory reaction that causes cells tissue damage (Peton and Le Loir, 2014). *S. aureus* pathogenicity depends on a wide variety of surface-based and secreted virulence factors collectively modulating tissue adherence, cytopathology and immune evasion (Paul *et al*, 2018).

There were several methods that can be used to screen for and differentiate *S. aureus* from other species. Whereas, when MSA was cultured by bacteria, it can worked on fermentation of mannitol sugar with the production of an acid in addition to that change of color as an indicator as it was phenol red, the color changed from red to yellow (D'Souza and Baron, 2005). The distinction of *S. aureus* from other staphylococci was