Molecular investigation of NUC and MECC genes in S. aureus isolated from meat

Ola Abdulwahhab Muslim[1]
[1]Karbala University / College of Medicine/Iraq (8111)

Abstract
Background and Aim: S. aureus was a pathogen which caused numerous infectious diseases, in human and animal. The current study was directed to investigate the occurrence of S. aureus that isolated from meat, then PCR detection of nuc and mecC genes in all isolates of S. aureus. Materials and Methods: An entire of 100 meat samples were investigated. The S. aureus isolates after identified were subjected to PCR assay to detect the nuc and mecC genes in all isolates of this bacteria. Results: The occurrence of S. aureus was 40% (40/100) that isolated from meat samples. Out of 40 samples of S. aureus studied by PCR technique, the results revealed that 100% of isolates give positive results of nuc gene, while no one isolates had mecC gene. Conclusion: The using of nuc gene-based PCR was an accurate and quick method for detection the S. aureus.
Keyword: nuc, mecC, PCR, S. aureus.

1. Introduction

S. aureus was incriminated in furthermost food toxicity outbreaks (Akineden, *et al.*, 2008). This bacteria was considered to be an essential source for humanity contagion that caused food-poisoning worldwide (Otto, 2010). However, the great quantities of antimicrobial resistance to *S. aureus* constituted a critical civic health risk and approximately 13–74% of *S. aureus's* contagions were defined as methicillin resistance *S. aureus* (MRSA) (Narayanan *et al.*, 2019).

A *S. aureus* found in throats, nasal passageways, hair, and skin of closely 50% of healthy persons. The food handlers were, consequently, assumed to be a potential causes of contamination for food, whichever directly or indirectly (Fisher, *et al.*, 2018). Also, this handlers performance as vehicles for human infection and may, therefore, pose a public health threat (Garciaa *et al.*, 2019). The signs of staphylococcal food poisoning, like abdominal cramps, nausea, vomiting, and diarrhea, occur 2-4 h following the ingestion of contaminated foods (Daum, 2018). The usage of antimicrobial drugs in humans and animals was poorly controlled, leading to the development of drug resistance. Multidrug-resistant bacteria, including *S. aureus* were therefore, frequently isolated from both humans and animals (Diab *et al.*, 2019).

One of all β-lactam antibiotics resistance amongst MRSA isolates, furthermore; was clarified via carrying mecA gene which locates on SCCmec (Yang *et al.*, 2016). The SCCmec XI was detected in UK and Denmark among cattle (from mastitis cows) and humans which carries mecC gene and the mecC was identified later as mecA LGA251 (Butaye *et al.*, 2016).

Moreover, *S. aureus* produces a thermostable extracellular nuclease (thermonuclease/TNase) encoded by the *nuc* gene, which is a virulence factor and one of the essential characteristics. Therefore, it can be used to differentiate *S. aureus* from *Staphylococcus* spp. The *nuc* gene is frequently used as a specific target for identifying *S. aureus* through PCR (Olson *et al.*, 2013). The use of *nuc* gene-based PCR which encode thermo nuclease is a very common use and can identify all isolates of *S aureus* from intramammary infection faster than conventional culturing methods. There is a clear need for strict hygienic measures, to reduce the risk of bacterial contamination (Javid *et al.*, 2018). The current study was aimed to PCR investigate of *nuc* and *mec*C genes in *S. aureus* which isolated from meat.

2. Materials and Methods

Identification of bacterial isolates

One hundred swabs were collected from meat from different supermarket of Thi-Qar province, during the period from March to August, 2022 by moistened sterile swabs with normal saline, these swabs directly inoculated on mannitol salt agar (MSA) (LAB/ United Kingdom) and incubated at 37°C for 24 hour; the identification of S. aureus was done by morphological examination followed by biochemical tests such as catalase test, oxidase test, detection of hemolysis, coagulase test, and DNase activity (Harley and Prescott, 2002).

Preparation of Chromosomal DNA

The chromosomal DNA extraction was carried out on entirely S. aureus isolates using Genomic DNA Extraction kit (Geneaid/Korea).

PCR diagnosis of *mec*C and *nuc* genes

The specific primer pairs of *mec*C gene: forward: 5′-GAA AAA AAG GCT TAG AAC GCC TC -3′ and reverse: 5′-GAA GAT CTT TTC CGT TTT CAG C-3′ (Stegger *et al.*, 2012). While for *nuc* gene was: forward: 5′-GCTTGCTATGATTGTGGTAGCC -3′ and reverse: 5′-TCTCTAGCAAGTCCCTTTTCCA-3′(Wongboot *et al.*,2013). PCR amplifications were performed in a total volume of 20μL and included: 3 μL of target DNA, 5μL of Mastermix, 1 μL of each

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forward and reverse primer and the volume completed to with DW.

The PCR cycling conditions of nuc gene: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 40 sec, annealing at 58°C for 35 sec, extension at 72°C for 35 S and final extension 72 for 5 min (Wongboot et al.,2013). Whereas for mecC gene: initial denaturation at 94°C for 15 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 1 min, extension at 72°C for 1 min and final extension for 10 min after the last cycle (Doğan et al., 2016). Electrophoresis of PCR product was carried out in 1.4% agarose gel and the presence of a 423 bp and 138 bp and indicate a positive result for nuc and mecC genes, respectively.

3. Results and Discussion

The results of present study documented that (40/100; 40%) of isolates identified as *S. aureus* after identification by morphological characteristics on culture media and biochemical tests. Then those isolates were additional characterized by amplifying (*nuc*) gene. The *Staphylococcus* found in air, dust, equipment, food, environmental surfaces, humans and /or animals (FDA, 2012). There were numerous kinds of Staphylococci, nevertheless the greatest infections were caused by *S. aureus* that was the third furthermost common cause of food poisoning in the world (Acco *et al.*, 2003). Hassan *et al.*, (2018) concluded that the meat products were careful and good medium for the growth of *Staphylococcus* and the toxins production.

The results of recent study was nearness with some studies like: Hassan *et al.*, (2018) and Shawish & AL-Humam (2016) recorded that the incidence of *S. aureus* was 38% in minced meat. Other studies revealed higher percentage of *S. aureus* such as Mousa *et al.*, (2014) (68% in beef burger) and Tarabees *et al.*, (2016) (70% in minced meat). While the study by Nadim, (2016) recorded that nearness percentage of *S. aureus* (36% in kofta). The existence of the *nuc* gene was used to identify *S. aureus*; and the agarose gel electrophoresis of nuc gene was shown in Fig 1.

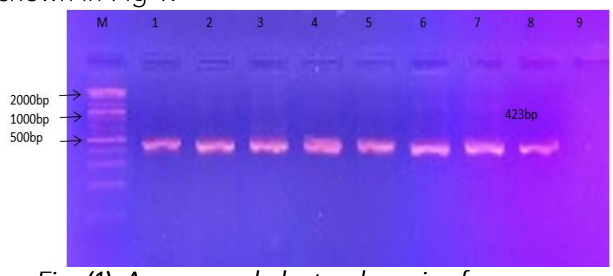

Fig. (1): Agarose gel electrophoresis of nuc gene. Where M: DNA marker ,1 – 8 was positive results and 9: as control negtive

Also, the *nuc* gene was discovered in most *S. aureus* isolates; nevertheless, certain isolates had been discovered that lack this gene. The different studies identified *nuc* gene in clinical and raw milk samples from dairy as positive for the *nuc* gene of 84% and 100%, respectively (Karmakar *et al.*, 2016). The *nuc* gene was known as a specific virulence factor of *S. aureus* (Andrade *et al.*, 2021), and it contributed to biofilm formation (Yu *et al.*, 2021) and immune evasion (Sultan *et al.*, 2019).

The PCR amplification of the nuc gene was used for accurate identification of S. aureus (Blaiotta et al., 2004). The current data was disagreed with study performed by Tamarapu et al., (2001) recorded that not completely S. aureus isolates gave positive results to the amplification of the nuc gene.

PCR results of nuc gene in present study disagreed with results of Juwita et al., (2022) revealed that only 32/56 (57.1%) of S. aureus samples harbored the nuc gene. While Sheet et al., (2022) showed that the nuc mRNA was identified in 12% of the isolates. The results of PCR amplification to detect the mecC gene was responsible for methicillin resistance showed that no isolates of S. aureus (0%) had this gene. While the entirely isolates of S. aureus (40; 100%) harbored nuc gene. The PCR results of percentage mecC gene was incorporated with other studies which recorded no isolates of S. aureus had the goal gene, such as the study by Sangarifar et al., (2017) documented that all S. aureus isolated failed to detect mecC gene. Also, the MRSA isolates harboring mecC gene were infrequently identified in human infections (Sabat et al., 2012). Most of mecC carrying strains in Denmark were found in rural areas; the study included of 22 patients, it was confirmed that (4/22) of patients had substantial animal contact (Petersen et al., 2013). In a study done by Schaumburg et al., (2012) in Germany, mecC was detected in only 2 isolates out of 3207 MRSA strains (0.062%). Amongst 8757 isolates of S. aureus isolated from mastitis, only 4 (0.046%) were identified as mecC carrying MRSA (Unnerstad et al., 2013). Nevertheless, a study performed in the Western Swiss displayed that 565 of MRSA isolates not had mecC gene (Basset et al., 2013). The colonization of MRSA poses a substantial threat for the hospital environment, resulting in nosocomial infections (Khan et al., 2007).

The current results was dissimilar with locally study that examined 22 isolates of MRSA, 22 to detected the present of mecC; there showed 30% of isolates had this gene (Degaim et al., 2019). The percentage of mecC gene in present study was differenced from the results of Stegger et al., (2012) exhibited that (12/203; 6%) isolates had mecC gene. Also, The current results recognized no percentage of mecC gene in S. aureus isolates, while Peterson et al., (2013) described that this gene established only in 1.5% of S. aureus isolates, the frequency of this gene increased and reached to 1.9% in 2010 and 2.8% in 2011 in the Denmark. The mecC gene was more detected in S. aureus isolated from animal samples and less frequently detected in humans, but Doğan et al., (2016) recorded that a mecC gene was not present in completely isolates of S. aureus. Likewise, the mecC gene was no detected in MRSA isolates (Alghizzi & Shami, 2021). The mecC gene source was not tacit adequately (Basset et al., 2013), but

Figueiredo & Ferreira, (2014) was suggested that the relations between humans and livestock had been maintained, and an incidence of mecC gene cross-transmission between the last populations.

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