ORIGINAL ARTICLE

Isolation and Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) From Different Sources of Food in Basra Province / Iraq

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ABSTRACT

Background: *Staphylococcal food-borne illness (SFD) is a food-borne infection that is highly common around the world. MRSA is a major cause of infections and poses a significant global health threat due to the toxins it can produce in food.* ***Objective:*** *is to characterize S. aureus isolates from foodstuffs collected from Basrah.* ***Methodology:*** *A total of 135 food samples of locally made food products were collected from different areas of Basrah markets in February 2022. The samples included (milk, cake, cheese), and various types of meats (beef, chicken, lamb).* ***Results and Discussion:*** *showed the presence 27 strains of S. aureus in percentage of 20% of the diverse samples (135 samples). 10.7% of the meat samples, 6.2% of the milk samples, 1% of the cake samples, and 2.1% of the cheese samples all had S. aureus. Using the Polymerase Chain Reaction (PCR), a total of S. aureus isolates from different food products were collected and identified. The detection of the mecA gene identified MRSA isolates. PCR amplification of the 27 phenotypically S. aureus isolates revealed that 4 were found to carry mecA genes in a percentage of (14.81%), exhibited resistance to methicillin, and were identified as (MRSA). The following amplicon sizes were revealed by the gel electrophoresis results: (DNA fragments) 756 bp, 500 bp (16S rDNA gene of S. aureus), and 310 bp (mecA gene).* ***Conclusion:*** *our results suggest that artisanal food producers should have better control production hygiene measure because it represents a potential risk to public health.*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a significant food-borne pathogen, raising concerns about its impact on public health. The isolation and genetic identification of MRSA strains from food sources play a crucial role in understanding their epidemiology, transmission dynamics, and antimicrobial resistance patterns [1].

The adaptable pathogen *S. aureus* can cause a wide range of infections, such as systemic illnesses, food poisoning, and infections of the skin and soft tissues [2]. But a significant obstacle to efficient treatment is the rise of MRSA strains, which are resistant to several kinds of antibiotics[3]. Therefore, isolating and identifying MRSA strains in food samples are crucial for comprehensively understanding their genetic characteristics and devising appropriate control measures.

The presence of MRSA in the food supply chain has been a growing concern due to its potential transmission to humans through contaminated food products [4]. Improper handling, processing, and storage practices in food preparation establishments can contribute to disseminating MRSA [5]. Therefore, the isolation and genetic identification of MRSA in food samples are critical for identifying potential sources and routes of transmission.

Genetic identification techniques, such as whole-genome sequencing, have revolutionized the study of MRSA strains by providing detailed insights into their genomic profiles and resistance mechanisms [6]. By analyzing the genetic sequences, researchers can identify specific genetic markers associated with antimicrobial resistance and pathogenicity, which are crucial for effective surveillance and control strategies. Understanding the genetic diversity of MRSA strains in food samples is essential for determining their origins and assessing their potential impact on public health [7]. Additionally, novel resistance genes or mobile genetic elements that aid in the spread of MRSA in the food industry can be found through genetic identification [8]. These results can direct the creation of focused initiatives to stop MRSA from spreading and reduce the danger of food-borne illnesses.

The isolation and genetic identification of MRSA strains from food sources are vital for understanding their genetic characteristics, transmission dynamics, and antimicrobial resistance patterns. Through the application of sophisticated molecular methods, scientists can learn more about the genetic diversity, origins, and pathogenic potential of MRSA in the food sector. This knowledge will contribute to developing effective surveillance and control strategies to mitigate the risks associated with MRSA as a food-borne pathogen. The aim of study characterize *S. aureus* isolates from foodstuffs collected from different areas of Basrah markets.

METHODOLOGY

Sample Collection and Isolation Bacteria

A total of 135 food samples of locally made food products were collected from different areas of Basrah markets in February 2022. The samples included milk (n = 25), cake (n = 15), cheese (n = 20), and different types of meats (beef, chicken, lamb) (n = 55). Samples of milk, cheese, meat, poultry, and lamb were collected using sterile cotton swabs. The swabs were gathered and placed in plastic screw-top tubes with a capacity of 10 mL of 0.1% (w/v) peptone water. The tubes were kept chilled while being transported to the Department's laboratory. Then were incubated for 24 hours at 37 °C, the samples were chosen based on their blackish hue. They were then infused into sterile tubes holding 5 milliliters of Brain Heart Infusion Broth-BHIB (HIMEDIA/India). After that, they were moved into the laboratory and kept at 37 ˚C for 24 hours. There are various ways to identify *S. aureus* and MRSA *S. aureus* strains, including morphological and genotypic characteristics. All samples were brought to the laboratory at 4°C.

Biochemical Tests

After the appearance of growth in BHIB, it was inoculated onto the following media: Mannitol salt agar -MSA (HIMEDIA/India) and MRSA Staphylococcus Chromogenic agar media (CONDA Pronadisa/Spain).

The colonies were allowed to grow for 24 hours at 37 ˚C. After incubation the isolated colonies were characterized by biochemical tests [9,10] . Tests for the fermentation of carbohydrates, including lactose, mannitol, sucrose, xylose, maltose, and trehalose, by adding phenol-red, which serves as a pH indicator.

When the medium's color transitioned from red to yellow, the fermentation of carbohydrates had good consequences. Catalase, Oxidase, Urease, Coagulase, DNase, Hemolysis, Motility, and Voges-Proskauer (VP) tests are additional tools that can be used in the fermentation of carbohydrates.

API Staph kit

The API Staph test strip consists of twenty capsules containing dehydrated biochemical media for colorimetric testing. As directed by the manufacturer, the tests were conducted and the results interpreted (API Staph, bioMérieux, France). In short, 6% sheep blood was added to agar plates used to reculture the frozen isolates (HIMEDIA/India). Following a 24-hour incubation period, 0.5 McFarland bacterial suspensions were made in 6 milliliters of sterile distilled water and added to the API test strip's wells. The strips were visually examined following a 24-hour incubation period at 37 ˚C.

Genotypic method

Genomic DNA from complete, freshly cultivated bacterial cells can be efficiently purified using the Presto ™ Mini Genomic DNA Bacteria Kit (Geneaid, China). DNA is able to attach to the glass fiber matrix of the spin column by means of lysing cells and breaking down protein using Lysis Buffer and chaotropic salt. A low salt Elution Buffer, TE, or water are used to elute the purified genomic DNA after contaminants were eliminated using a Wash Buffer that contains ethanol. Without the need for alcohol precipitation or phenol/chloroform extraction, the entire process can be finished in 25 minutes. The 20–30 kb pure DNA is appropriate for PCR and other enzymatic processes.

DNA Extraction

The Presto ™ Mini Genomic DNA Bacteria Kit was utilized to extract fresh bacterial genomic DNA of Staphylococcus spp. from nutrient agar samples in accordance with a previously published protocol [11] . The isolated DNA was verified using a Nanodrop spectrophotometer and refrigerated at 20°C until needed.

Polymerase chain reaction (PCR)

Molecular confirmation of *Staphylococcus spp*. was carried out by PCR analysis on the isolated *Staphylococcus* strains. The boiling process was used to manually extract all of the DNA [12] . For the PCR, a 30 µl reaction mixture was prepared, comprising 15 µl of 2x Master Mix (Biolabs), 1.5 µl of forward primer (5'-AACTCTGTTATTAGGGAAGAACA-3'), 1.5µl of reverse primer (5′- CCACCTTCCTCCGGTTTGTCACC -3′), and 3 µl of DNA. The amplification was conducted in a thermocycler (MultiGene, Labnet International, Inc.). The amplification technique comprised 35 cycles, each of which included denaturation at 96°C for 45 seconds, annealing at 54°C for one minute, and extension at 72°C for one minute. The first cycle was completed at 96°C for five minutes. Finally, the amplification process was completed with a 5-minute extension step at 72°C. By comparing BLAST National Center for Biotechnology Information databases, sequencing data were examined using the MEGA X program.

To determine the presence of MRSA, the *mecA* gene was targeted using *Mec*A1 (5'-GTAGAAATGACTGAACGTCCGATAA-3') and

*MecA*2 (5'-CCAATTCCACAATTGTTTCGGTCTAA-3') primers. For amplifying the *mecA* genes, Throughout the thermocycling procedure, there was an initial denaturation stage at 95°C for three minutes, thirty cycles of denaturation at 94°C for one minute, annealing at 53°C for thirty seconds, extension at 72°C for one minute, and a final extension at 72°C for six minutes [13] .

RESULTS

Figure 1 shows that the bacterial test of the suspected *S. aureus* in MSA media produced positive results. The medium's color changed from red to yellow, and the colony form was gold-yellow., large circular and low raised. At this step 55 suspected isolates of *S. aureus.*

Fig. 1: The typical isolate of *S. aureus* on MSA medium

S. aureus's carbohydrate fermentation test could ferment sugars such as sucrose, trehalose, and maltose, as seen by the sugar medium turning yellow. Due to their inability to undergo fermentation, mannitol, lactose, and xylose would continue to be red. On the other hand, VP would turn red when it came in contact with potassium hydroxide (reagent B) and alpha-naphthol (reagent A) (Figure 2, Table 1). From this step obtained 35 suspected isolates of *S. aureus*.

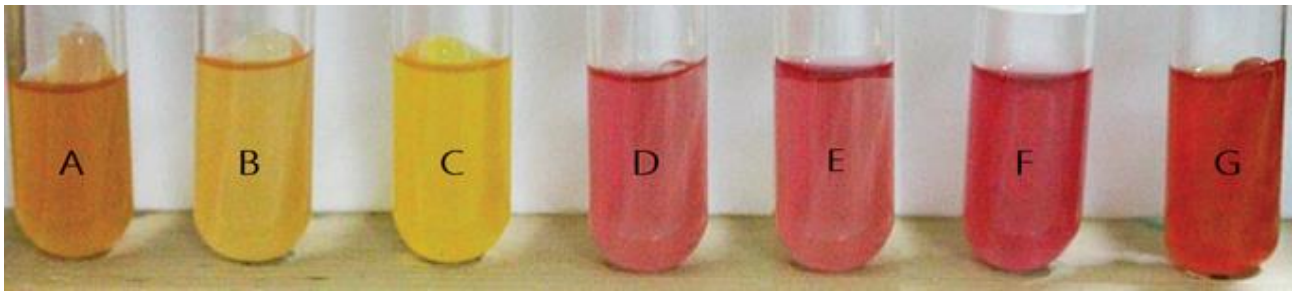

Fig. 2: Carbohydrate fermentation test to shows *S. aureus*. Description: (A) Maltose broth, (B) Trehalose broth, (C) Sucrose broth, (D) Xylose broth, (E) VP broth, (F) Lactose broth and (G) Mannitol broth.

Table 1: The biochemical tests of *Staphylococcus aureus*

Bacteria name	Namper isolate	Maltose	Trehalose	Sucrose	Xylose	VP test	Lactose	Mannitol	Catalase	Oxidase	Urease	Coagulase	DNase	Hemolysis	Motility
S. auruase	35	+	+	+	–	+	–	–	+	-	+	+	+	±	-

The type of Staphylococcus sp. isolates was determined by using the API Staph test as a confirmation test. The results of API Staph demonstrate that D-Glucose, D-Fructose, D-Mannose, Maltose, Lactose, Trehalose, D-Mannitol, Xylitol, D-Melibiose, and Potassium nitrate may all be fermented. (Figure 3). aphthyl-acid phosphate, sodium pyruvate, rafinose, xylose, sucrose, methyl-D-glucoside, N-acetyl-glucosamine, arginine, and urea; nevertheless, they result in unfavorable outcomes for xylitol and melibiose, as seen by failure to change color into yellow. Following the acquisition of color change reaction data from API Staph, API Web was used assessing the data. Using the API-Staph technique, 30 isolates were used in this investigation and were determined to be *S. aureus* with an ID percentage of 97.3–97.9%.

Fig. 3 : Detection of Staph-aureus by API Staph

When the DNA fragments from 27 isolates were examined under UV light, it was seen that the extracted DNA (100%) gave positive results. Specifically, a single band at position 756 bp was observed on the agarose gel when visualized under ultraviolet (UV) light and compared to the DNA ladder, as shown in (Figure 4). Out of the total isolates, only 27 (20%) were identified as *S. aureus* based on the PCR analysis, which particularly detected the 16S rDNA gene. The results were positive, indicating the presence of a single band at position 500 bp (Figure 5).

The isolates were distributed as follows: 14 (10.7%) meat samples, 3 (6.2%) milk samples, 1 (1%) cake sample and (2.1%) cheese sample. Furthermore, 4 out of the 27 isolates (14.81%) exhibited methicillin resistance and were identified as MRSA. The identification of the *mecA* gene, a genotyping marker, in a single band at position 310 bp served as confirmation that the MRSA was real. The remaining three isolates contained *S. aureus* that was susceptible to methicillin (Figure 6).

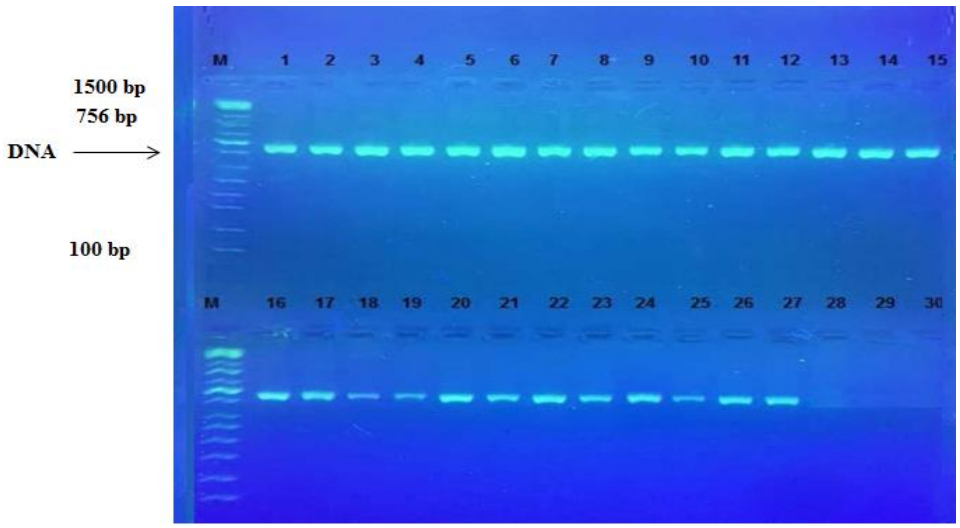

Fig. 4: DNA fragments under UV light

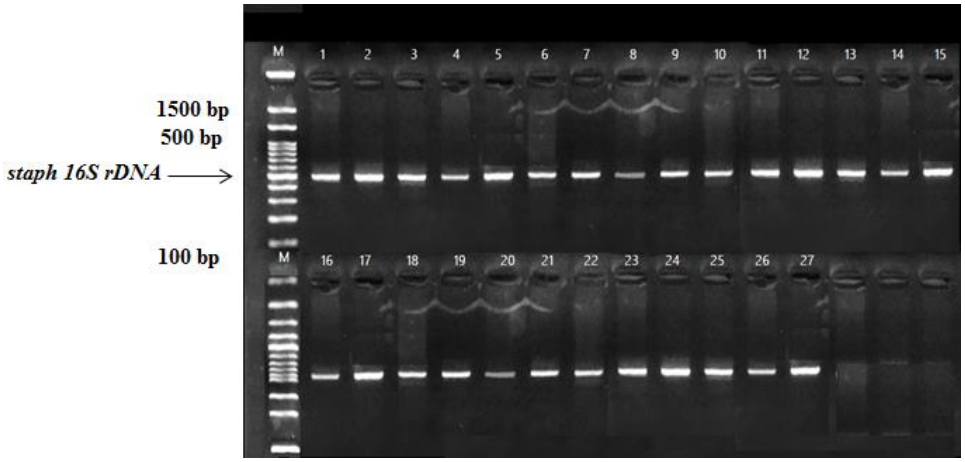

Fig. 5: PCR amplification of 16S rDNA gene of *S. aureus*

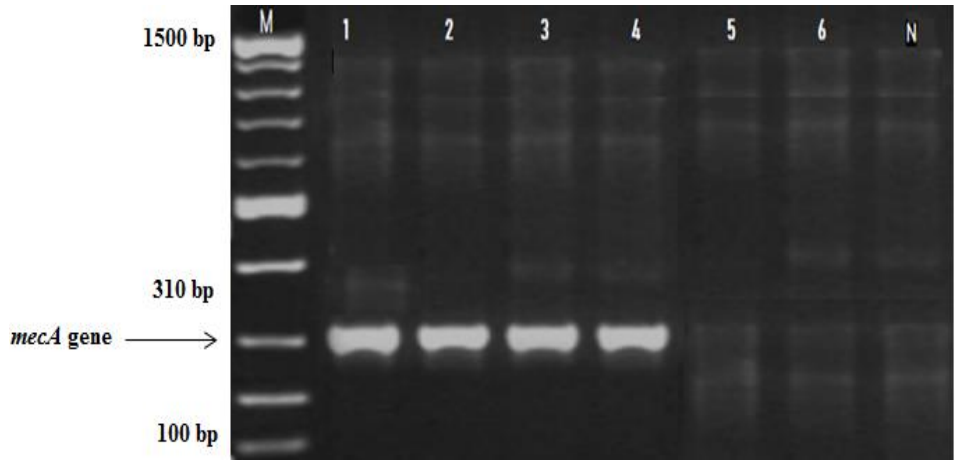

Fig. 6: PCR amplification of *mecA* gene of *S. aureus*

DISCUSSION

One of the most frequent causes of food-borne illnesses (FBD) globally is thought to be staphylococcal food poisoning (SFD). The main source of contamination, according to investigations into outbreaks, is improper handling of cooked or processed food. While *S. aureus* can be eliminated by heat treatment and competition with other bacteria in pasteurized and fermented foods, the toxins that *S. aureus* produces are still heat-resistant and can cause SFD [14] .

The growth of *S. aureus* from the sample of dairy products (milk, cake, cheese) and animal meat (beef, poultry, lamb) was demonstrated by the results. The growth of *S. aureus* indicates contamination, which is probably the result of the unhygienic handling of the products and animal meat [15]. However, *S. aureus* could ferment media of lactose, maltose, sucrose, trehalose, VP, galactose, and mannitol. The biochemical confirmation test of *S. aureus* using API Staph had an ID percentage from (97.3- 97.9%) these results were similar to (Kloos and Wolfshohl, 1982[16] ; Raheema and Abed, 2019[17]) . However, in this study, we found *S. aureus* in food samples from local sources with a lower percentage than in previous studies [18,19]. This may be due to the difference in several factors, such as the type of samples (e.g., animal- or non-animal-derived foods), sampling site (e.g., street-vendors or supermarkets), the sample size, the techniques used in manufacture (such as whether or not bactericidal temperatures are utilized), the identification methods (such as biochemical testing or molecular biology techniques), and the general hygienic measures employed throughout the preparation and handling of the foods. Specifically, *S. aureus* is a commensal bacteria that lives on human and animal mucous membranes, skin, and noses [20] . Recently, MRSA has already been isolated from the food product chain[21-23]. The majority of reports of this bacteria's presence included dairy products like milk and cheese as well as meats like beef, poultry, lamb, and turkey [24] . *mec*A gene is used to identify the methicillin-resistant in all *S. aureus* strains using PCR assay. Upon PCR

amplification of a 310 bp *mecA* gene fragment, three of the 27 MRSA isolates were found to be *mecA* negative. Previous studies reported that the *mecA* gene was detected in band 310 bp and that PCR assays were more rapid and accurate than conventional methods for the detection of MRSA strains [25-29] .

CONCLUSION

In conclusion, our study indicates that PCR assay is an easy and reliable tool for detecting MRSA in dairy products and various types of meats. Risk assessment and *S. aureus* contamination prevention must be taken into consideration, and they must be done quickly. Therefore, routine tests for detecting *S. aureus* and its toxins may not yield positive results; thus, modern diagnostic techniques and laboratory analysis of food products can help combat Staphylococcal food poisoning (SFD).

Ethics statement
The study does not require ethical approval, as it did not include humans or animals.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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