INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Cluster Analysis of Clinical, Food, and Handler Methicillin-Resistant Staphylococcus Aureus Isolates Characterized by Pulse Field Gel Electrophoresis

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OPEN ACCESS

Received: 04-01-2022

Accepted: 25-05-2022

Published: 08-11-2022

Citation: Abdulla TS, Fakhry SS, Jassin SA, Rashid FA, Abdulbaqi AAQ (2022) Cluster Analysis of Clinical, Food, and Handler Methicillin-Resistant Staphylococcus Aureus Isolates Characterized by Pulse Field Gel Electrophoresis. Indian Journal of Science and Technology 15(41): 2171-2181. https://doi.org/10.17485/IJST/v15i41.15

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Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: To identify and to characterize of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected from food (n=43), food handler (n=8), and clinical samples (n=15). **Methods:** Genotyping was applied for the purposes of epidemiological investigation and source tracking of these isolates. Pulsed field gel electrophoresis (PFGE) was performed using the restriction endonucleases SmaI. **Findings:** PFGE analysis showed that pulse types of isolates from clinical samples differed that from food and food handler isolates. However, a similarity was observed between pulse types of isolates from food and food handlers, suggesting an epidemiological association between isolates from these two sources and that MRSA may be transmitted from food handlers to foods. **Novelty :** PFGE genotyping of MRSA isolates was useful, as a first study of its type in Iraq, for investigating epidemiological relatedness and even contaminated food sources.

Keywords: Epidemiology; MRSA isolates; PFGE; SmaI

1 Introduction

The presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in medicine was reported shortly after methicillin was introduced into the clinic for the treatment of infections sustained by these microorganisms[(1)]. The ability colonizing and the active multiplication of staphylococci represent virulence characters. The contributing risk factors when the infection takes root are represented by the slow healing of some recurrent infections, by prolonged antibiotic treatments, by the use of catheters and again by the presence of wounds[(2)]. *S. aureus* is one of the most frequent agents responsible for mastitis in dairy cattle[(3,4)], in sheep and in the goat and is also often involved in skin infections of other animal species[(5)].

The *S. aureus* forms no spores, however, may result in contaminating the food products throughout the processing and preparation of the food[(6)]. It is capable of

growing in many different temperature values (7 - 48.5°C; optimal 30-37°C), pH (4.2 - 9.3; optimal 7-7.5), and concentration of the NaCl up to 15%. *S. aureus* is an organism with desiccation tolerance, which is capable of surviving in the possibly stressful and dry environments, like human skin and inanimate surfaces like the clothing [7]. In the case where the food providers do not abide by the hygiene rules, they are capable of transferring contamination to the foods. A 10^5 bacteria/gram concentration in the foods is adequate for producing the toxin and inducing of the illness [8,9]. Numerous researches showed that 15-80% of *S. aureus* which have been separated from a variety of the food sources (such as ice cream, dairy products and meats) [2,10–12] .

The contamination of the *S. aureus* in the food products may take place from environment throughout the processing and handling [13]. Incorrect practices of the food handling in retail food industries have been considered to be contributing to high numbers of the outbreaks of the [1,6]. The researchers showed that most outbreaks of the FBD are a result of those practices [4].

Sometimes, the *S. aureus* was related to the food intake and knowledge about the epidemiology and the spread was utilized for developing the strategies for the prevention of MRSA distribution. At the same time, the progression of a variety of the molecular typing methods emerged, with the aim at the detection of specific molecular properties or phenotypes of every one of the strains which are in question.

Pulse Field Gel Electrophoresis (PFGE) is viewed as gold standard for the *S. aureus* of the genotyping due to its efficiency (its reproducibility and discriminatory powers) and practice [3,4]. For the purpose of identifying epidemiologic properties of the isolates of the MRSA and more significantly, for studying the progression and the spreading of the epidemic clones, the CDC established a molecular network of the sub typing (Pulse Net) for the surveillance of the foodborne diseases and have created a national database of the *S. aureus* profiles to investigate the outbreaks of the MRSA and the global tracking of its strain type [6]. This study aimed at applying the PFGE-based method as a valuable tool used by epidemiological and source tracking study of MRSA isolates from various environments in Iraq, particularly those causing food poisoning.

2 Material and methods

2.1 MRSA isolates

MRSA isolates, which included 43 food, 8 food handler, and 15 clinical source isolates, were obtained from the Food Contamination Research Center, Baghdad, Iraq. For confirmation the identification of the 66 isolates were performed on the Vitek 2 automated system.

2.2 Pulse Filed Gel Electrophoresis

Chromosomal DNA was isolated as described by USA Centers for Disease Control and Prevention (CDC, 2007) [14] and subjected to restriction digestion with smaI restriction endonuclease (Bio-Lab, UK). using the CHEF Mapper PFGE system (Bio-Rad laboratories). *S. aureus* (Mu50 ATCC 700699) was used as reference strain. The PFGE band profiles were analyzed using the GelCompar II software (Version 5.0, Applied Maths, Sint-Martens-Latem, Belgium). The dendrogram was constructed based on the Dice similarity coefficients using the unweighted pair group method with arithmetic mean (UPGMA). The resulting dendrogram was verified based on the visual inspection of the banding patterns. The collected research data were interpreted in accordance with the established guidelines [13,14]. The isolates with an 80% band profile similarity were categorized under the same cluster and designated capital letters. The isolates in the same cluster were further categorized into a sub-cluster, were they to be 80% to 100% similar, and designated with the lowercase letter of the name of their cluster followed by a number. Identical (100% similarity) isolates were categorized into the same sub-cluster. Table 1 shows the steps of the PFGE that were used for the genotyping of MRSA isolates.

Table 1. The applied PFGE Pulse Net protocol

1	Prepare cell suspensions a- TE buffer b- Bacterial cell concentration	400 μl TE buffer final concentration (10 mM Tris-HCl , 1 mM EDTA, PH8) Absorbance of culture to attain Abs=0.9-1.1 at 610 nm
2	Prepare Pluges a- Agarose b-Lysostaphin	1.8% SeaKem Gold agarose in TE buffer (0.9% final plug concentration after cell suspension is added) Used 3 μl recombinant stock solution (sigma L-0761)
3	Cell Lysis	Removed each plug to sterile tube containing 3ml EC lysis buffer final concentration (6 mM Tris-HCl, pH 8, 1M NaCl, 100 mM EDTA, pH 8, 0.5% Brij-58, 0.2% Sodium deoxycholate, 0.5% Sodium lauroylsrcosine) incubation condition: at 37 °C for 4 hours
4	Plug washing	3 times with 4 ml TE buffer at room temperature for 30 min.

Continued on next page

Table 1 continued

5	Restriction Enzyme Digestion with SmaI	a- counterpoise the sliced plugs in water ¬buffer mix at normal temperature for (30-45) min, plug section that must be under water ¬buffer mix b- Incubated the sliced plugs with SmaI* (30 U per sliced pluge) in 25°C at least 3 hours
6	Running the gel	a- Running gel was prepared of (1.0%) Sea Kem Gold agarose in 0.5% TBE b- Running buffer (0.50X TBE) was prepared c-Electrophoresis: Block 1: State 1[6.0] v/cm a= linear, 11 h Ang=[+ 60] Initial switch time:5 s, Final switch time: 15s Block 1: State 2[6.0] v/cm a= linear Ang=[- 60] Initial switch time:5 s, Final switch time: 15s Block 2: State 1[6.0] v/cm a= linear, 11 h Ang=[+ 60] Initial switch time:15 s, Final switch time: 45s Block 2: State 2[6.0] v/cm a= linear Ang=[- 60] Initial switch time:15 s, Final switch time: 45s
7	Staining and destaining	Staining with ethidium bromide (50μl/500ml D.W) for 30 min. Destain gel for 30-40 min.

*30 minutes needed if using Promega enzyme. This is shortened to 15-30 minutes if using New England Biolabs enzymes.

3 Results and Discussion

3.1 Food isolates

Analysis of the entire set of 43 isolates by the PFGE was carried out with the use of restriction endonucleases SmaI-digested whole cell DNA**.** The outcomes revealed the presence of several distinct cleavage patterns from 43 isolates, which yielded between 8-18 restriction fragments for each isolate, ranging in size from less than 225 to 2200 bp (Figures 1, 2 and 3).

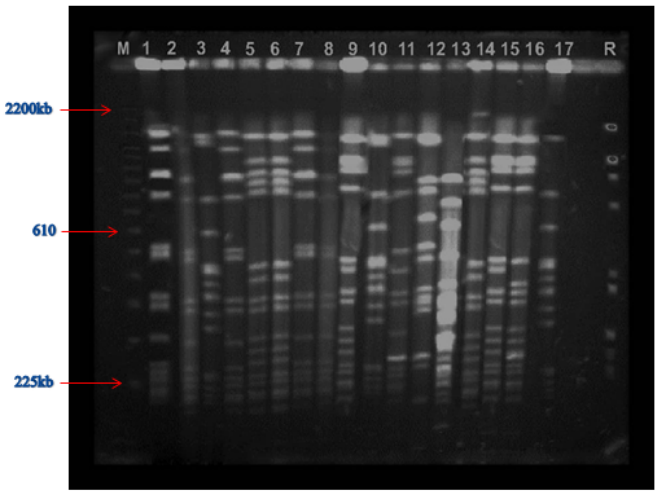

Fig 1. PFGE analysis of *S. aureus* isolates from food using restriction enzymes SmaI. Lane (M): DNA marker (Saccharomyces cerevisiae); Lanes (1-17): The isolates numbers (CM1, CM2, CE1, CE2, CP1, CR1, CR2, CR3, CR4, CR5, CK1, CMP1, CC1, CC2, CC3, MP1, MP2); Lane (R): Reference strain *S. aureus* (Mu50 ATCC 700699).

Genetic variations might occur due to point mutations in the sites of the restriction or be associated with insertion or deletion of mobile DNA elements in a subtype. These variations often cause minor differences, in the order of 2-3 fragments, in the banding patterns of the PFGE. Therefore, it is generally accepted that an isolate is interpreted as closely associated with an outbreak strain when the difference is about 2-3 fragments. They are interpreted as potentially associated when there is a 4-6 fragment difference and unrelated in the case where the difference is 7 or higher[(15)].

PFGE has been viewed as a golden standard approach for the typing of *S. aureus*, as a result of its very discriminative power, reliability for identifying strains at subspecies level, capability of determining inter-strain genetic association, and sufficient

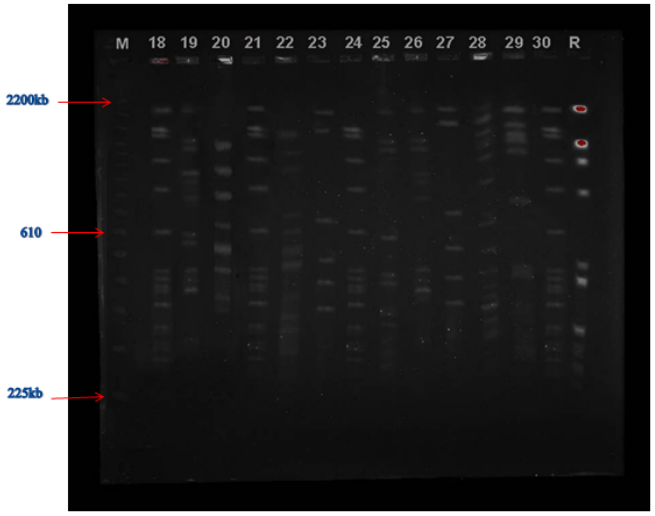

Fig 2. PFGE analysis of *S. aureus i*solates from food using restriction enzymes SmaI. Lane (M): DNA marker (Saccharomyces cerevisiae), Lanes (18-30): The isolates numbers (MP3, CA1, DP1, DP2, DP3, DP4, S1, S2, S3, S4, S5, S6, S7); Lane (R): Reference strain *S. aureus* (Mu50 ATCC 700699).

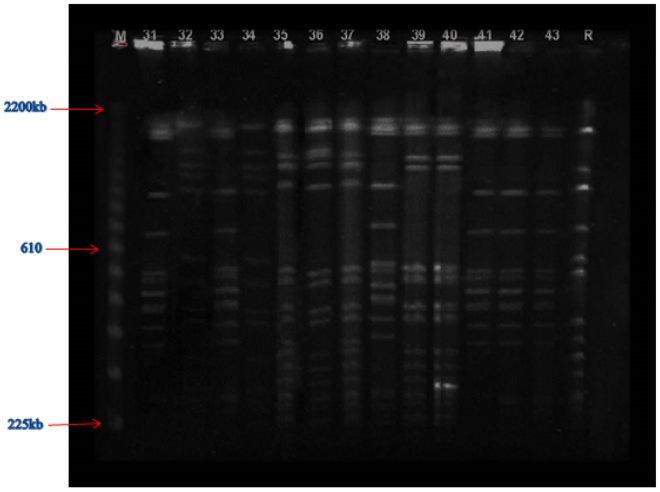

Fig 3. PFGE analysis of *S. aureus* isolates from food using restriction enzymes SmaI. Lane (M): DNA marker (Saccharomyces cerevisiae), Lanes (31-43): The isolates numbers (S8, S9, CE3, CC4, CC5, MP4, MP5, MP6, CA2, CA3, DP5, S10, S11), Lane (R): Reference strain *S. aureus* (Mu50 ATCC 700699).

correlation to the epidemiological data[16].

Nevertheless, PFGE is also time consuming, slow, and require sophisticated equipment and particularly trained personnel, which hampered its widespread use. In addition, the need for following exact standard protocols and agreeing on the ways for comparing the restriction patterns obtained in various labs was observed as a PFGE typing limitation[17].

3.2 Cluster analysis of genotyped food isolates

Patterns obtained by PFGE ranged from 9 to 15 well-resolved fragments of approximately 2250 to 220 kb) Figure 1-3,5 and 7). Percentage similarity among patterns was determined by cluster analysis in a dendrogram (Figure 4,6 and 8-9). Epidemiologically plausible groups (clusters) were identified using the cut-off of at least 80% similarity

Dendrogram was constructed according to Jaccard genetics similarity of matrix using UPGMA [18]

It showed similarity among the 43 isolates of *S. aureus* depending on the isolates and different food sources. The results revealed that the isolates of *S. aureus* are classified to 2 main groups, cluster I and cluster II, with 0.28 % similarity at 95% confidence level, as shown in (Figure 4).

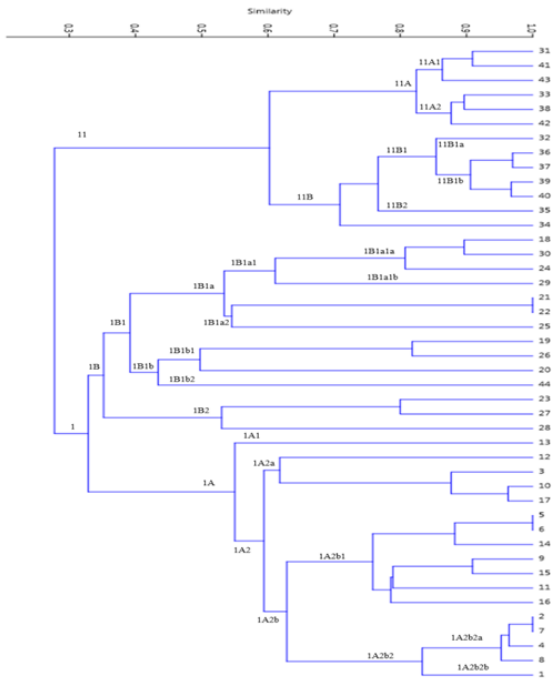

Fig 4. Dendrogram showing the percentage of genetic similarity between the 43 isolated from food samples,SmaI digestion PFGE patterns, with the use of the approach of the unweighted pair group with the arithmetic average values (UPGMA).

The main cluster I included the largest number of *S. aureus* isolates (30; 69.77%) from different food sources with standard strain. Cluster I was divided into two subclusters, IA and IB, with 33% similarity. Subcluster IA contained 13 (43.33%) isolates of *S. aureus* and it was divided into two subclusters, IA1 and IA2. The results revealed that the degree of similarity between the subcluster IA1, whichcontained one isolate(CC1), and the 16 isolates of subcluster IA2(CM1, CM2, CE2, CP1, CR3, MP1, CK1, CC3, CR4, CC2, CR1, CP1, MP2, CR5, CE1, CMP1) was 55% where the similarity was referred to all isolates of subcluster IA were classified to (CM1, CR3, CE2, CM2, CR2, MP1, CK1, CC3, CR4, CC2, CR1, CP1, MP2, CR5, CE1, CMP1). The subcluster IA2 was divided into two subclusters, IA2aand IA2b. A similarity value of 60% was recorded between the isolates of subcluster IA2a (MP2, CR5, CE1, CMP1) and the isolates of subcluster IA2b (CM1, CR3, CE2, CM2, CR2, MP1, CK1, CC3, CR4, CC2, CR1, CP1) which belonged to the (CM1, CR3, CE2, CM2, CR2, MP1, CK1, CC3, CR4, CC2, CR1, CP1).

According to the results of the molecular study, the isolates of subcluster IA2a (MP2, CR5, CE1, CMP1) showed a similarity of 62%. This subcluster was classified into two subclusters, where only one isolate (12) belonged to the subcluster 1A2a1, while the subcluster 1A2a2 consisted of 3 isolates (MP2, CR5, CE1) which belonged to the (MP2, CR5, CE1), which showed a similarity of 88%. From these isolates, only two isolates (MP2, CR5) showed a similarity of 86 %.

The subcluster IA2b was divided into two subclusters, IA2b1 and IA2b2. The percentage of similarity between the subcluster IA2b1, which consisted of seven isolates (MP1, CK1, CC3, CR4, CC2, CR1, CP1), and the isolates of subcluster IA2b2 (CM1, CR3, CE2, CM2, CK1) was 76 %, which is due to the fact that all these isolates were classified.

From the results, it was also observed that the degree of similarity between the isolates of subcluster IA2b2a (CR3, CE2, CM2, CR2) and subcluster IA2b2b, which consisted of one isolate (CM1), was 84%, depending on their classification to the (CR3, CE2, CM2, CR2). A high percentage of similarity (96%) was recorded between the two isolates (CE2, CR3) of subcluster IA2b2a which belonged to the (CE2, CR3) and the isolates (CM2, CR2).

The second subcluster IB included 17 (56.66%) isolates of *S. aureus* and reference isolate (*S. aureus*) it was divided into two subclusters, IB1 and IB2. The percentage of similarity between the subcluster IB2, which contained (DP4, S4, S5) isolates, and

the isolates of subcluster IB1 was 33%. Most isolates of subcluster IB were isolated from types of food that included vegetables salad and dairy products. There were also differences between the isolates MP3 and CA1, which were collected from meat products and cake, respectively.

The subcluster IB1 was divided into two subclusters, IB1a and IB1b. The degree of similarity between IB1b isolates (DP1, S3, CA1, 44 ref) and IB1a isolates (S2, DP2, DP3, S6, S1, S7, MP3) was 35%. The subcluster IB1a was divided into two subclusters, IB1a1 and IB1a2. The percentage of similarity between IB1a2 isolates (S2, DP2, and DP3) and IB1a1 isolates (S6, S1, S7, MP3) was 52%. The subcluster IB1a1 was divided into two subclusters, IB1a1a and IB1a1b. The results observed a similarity of 60% between the isolate of subcluster IB1a1b (S6) and the isolates of subcluster IB1a1a (S1, S7, MP3), where all of these isolates belonged to the vegetables salad. Also, the results showed that the degree of similarity between the two isolates (DP2, DP3) which belonged to dairy product was 100%, but they had similarity of 55% with isolate S2 from vegetables salad in subcluster 1B1a1b.

The subcluster IB1b was divided into two subclusters, IB1b1and IB1b2. The percentage of similarity between the isolate of subcluster IB1b2 (DP1) collected from dairy product and the isolates of subcluster IB1b1 (S3) collected from salad and CA1 collected from cake was 49%.

In addition, cluster II included 13 (26.53%) isolates from different food sources, including cooked food, dairy products, vegetables salad, meat products, and pastry. Cluster II was divided into two subclusters, IIA and IIB, with percentage of similarity of 60%. The results showed a similarity of 82% among the isolates of subcluster IIA, which was divided into two subclusters, IIA1 and IIA2. The degree of similarity between the isolates of subcluster IIA1 (S8, DP5, S11) was 87%. On the other hand, subcluster IIA2 contained three isolates (S10, MP6, and CE3) with a similarity of 88%. The isolates of the second major subcluster IIB (CC4, CC5, CA3, CA2, MP5, MP4, and S9) were similar in 71%, and it was divided into two subclusters, IIB1 and IIB2, with similarity of 76%.

The subcluster I1B1 was divided into two subclusters, I1B1a and I1B1b. The degree of similarity between the isolates of subcluster IB1b (CA2, CA3) and subcluster IB1a (MP4, MP5) was 90%.

3.3 Food Handler isolates

PFGE analysis of restriction endonuclease-digested whole cell DNA revealed the presence of several distinct cleavage patterns with SmaI, which yielded between 10 and 12 restriction fragments ranging in size from 270 to 1600 bp. Of these bands, 10 fragment lines were polymorphic and 2 monomorphic, with molecular weight that approximately ranged 825 – 945 bp in comparison with the standard strain of *S. aureus* (Mu50 ATCC 700699) which appeared with 10 fragment lines, as shown in (Figure 5).

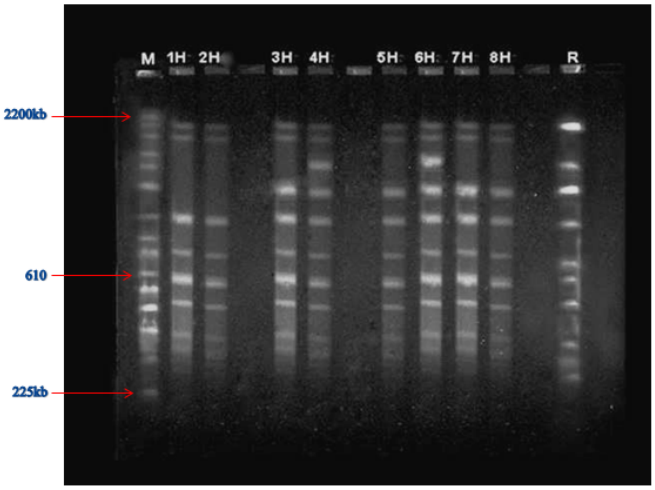

Fig 5. PFGE analysis of *S. aureus* isolates from food handler using restriction enzymes SmaI. Lane (M): DNA marker (Saccharomyces cerevisiae), Lanes (1-8): The isolates numbers, Lane (R): Reference strain *S. aureus* (Mu50 ATCC 700699).

3.4 Cluster analysis of genotyped food handler isolates

A dendogram was constructed according to Jaccard genetics similarity of matrix using UPGMA analysis[18], which showed the similarity among 8 isolates of *S. aureus* depending on the isolates. The outcomes revealed that the isolates of *S. aureus* were classified to 2 main groups, cluster I and cluster II, with a similarity of 93 % and confidence level of 95%**.**

The relatedness of the patterns is illustrated in dendrogram (Figure 6). The main cluster I included the largest number of *S. aureus* isolates (6, 75%) from food handler. Cluster I was divided into two subclusters, IA and IB, with 95% similarity. '

The subcluster IA contained 4 isolates (H3, H4, H5, H8) which were similar by 97 %, while each two isolates were similar in 100%. The subcluster IB also had two isolates (H4, H6) which were similar by 100%. Whereas the similarity between the two isolates of H1 and H2 of the main cluster II was 100%.

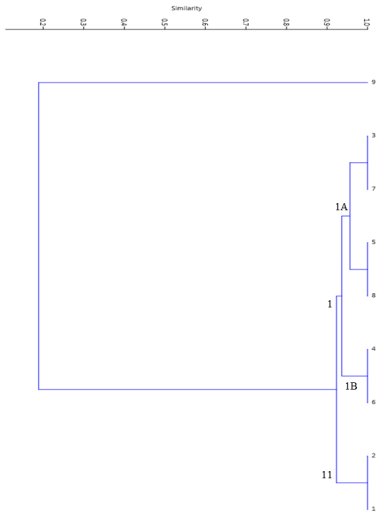

Fig 6. Dendrogram showing the percentage of genetic similarity between the 8 isolated from food handler samples, SmaI digestion PFGE patterns, with the use of the approach of the unweighted pair group with the arithmetic average values (UPGMA).

3.5 Clinical isolates

Analysis of the 15 *S. aureus* isolates that have been isolated from clinical sources (burn swabs) demonstrated the presence of several distinct cleavage patterns with SmaI, which yielded between 15 and 17 restriction fragments ranging in size from 225 to 2200 bp. Of these bands, 15 fragment lines were polymorphic and 2 were monomorphic, with molecular weight that approximately ranged 680 – 750 bp in comparison with the standard strain of *S. aureus* (Mu50 ATCC 700699) which appeared with 10 fragment lines, as shown in (Figure 7). This is an indication of a considerable diversity among the isolates associated with sporadic cases. These results agree with those of other researchers, which also found considerable genetic diversity for S. aureus[19–22].

3.6 Cluster analysis of genotyped clinical isolates

A dendogram was constructed according to Jaccard genetics similarity of matrix using UPGMA analysis[18], which showed the similarity among 15 isolates of *S. aureus* depending on the comparison with the standard strain of *S. aureus* (Mu50 ATCC 700699).

The findings shown in (Figure 8) reveal that the isolates of *S. aureus* were classified to 2 main groups, which are cluster I and cluster II, with a similarity of 71 %. In addition, the standard strain formed another group, which is cluster III, with similarity of 34 % and confidence level of 95%. The main cluster I included 8 isolates (53.33%)and was divided into two subclusters, IA and IB, with 71% similarity. The subcluster IA contained 2 isolates (CL13, CL15) of S. aureus with similarity of 94%. The results revealed that the degree of similarity between the subcluster IB1 whichconsisted5 of isolates (CL2, CL3, CL8, CL14, CL11) and the one isolate (CL1) of subcluster IB2was 80%. The subcluster IB1 was divided into two subclusters, IB1aand IB1b.

A similarity of 92% was found between the isolates of subcluster IB1a1 (CL2, CL3), whereas the value was 97% between the isolates of subcluster IB1a2 (CL8).

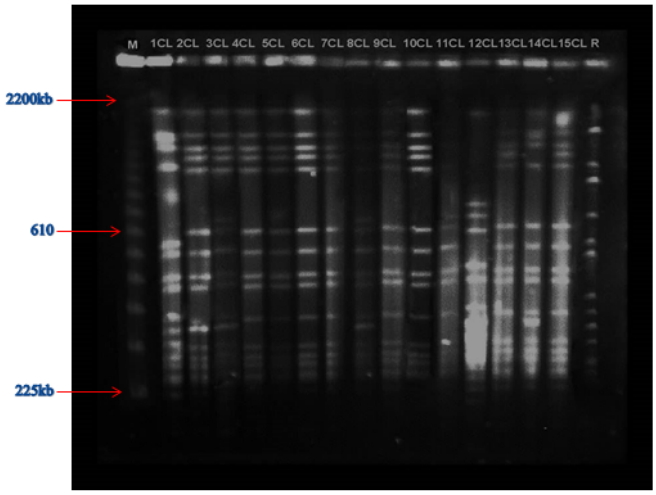

Fig 7. PFGE analysis of *S. aureus* isolates from clinical samples using restriction enzymes SmaI. Lane (M): DNA marker (Saccharomyces cerevisiae); Lanes (1-15): The isolates numbers, Lane (R): Reference strain *S. aureus* (Mu50 ATCC 700699).

According to the results of the molecular study, the isolates of cluster I1 showed a similarity of 90% and classified into two subclusters; 11A which consisted of 4 isolates with a similarity of 96%and 11B which contained 2 isolates (CL5, CL10) with a similarity of 94%. On the other hand, the sub cluster IIA contained three isolates and was divided into two subcluster. The subcluster 11A1 showed a similarity of 100% between its two isolates (CL4, CL7), while the subcluster 11A2 showed a similarity of 97 % between its two isolates (CL6, CL9). The findings of the current study demonstrated a high similarity between S. aureus isolates collected from the same source (burn swab samples).

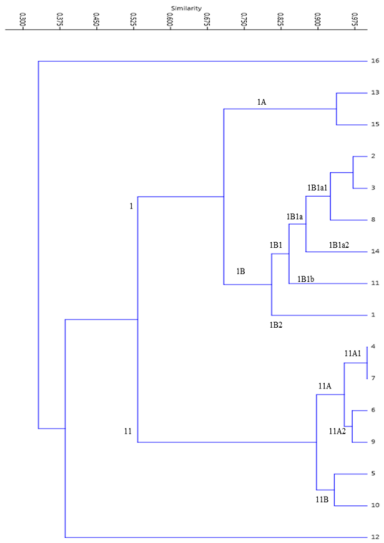

Fig 8. Dendrogram showing the percentage of genetic similarity between the 15 isolated from clinical samples,SmaI digestion PFGE patterns, with the use of the approach of the unweighted pair group with the arithmetic average values (UPGMA).

3.7 Cluster analysis of *S. aureus* isolated from different sources

The outcomes of UPGMA for *S. aureus* isolated from the three types of sources showed similarities among the 66 isolates of *S. aureus* depending on the isolate and the sources of isolation [different food samples, food handler (nose swabs), clinical

specimens (burns swabs)] and standard strain (*S. aureus* Mu50 ATCC 700699). The findings indicated that the isolates of S. aureus were classified to 2 main groups, which are cluster I and cluster II with a similarity of 25 % and confidence level of 95%. The relatedness of the patterns is illustrated in the dendrogram shown in (Figure 9). The main cluster I included the largest number of *S. aureus* isolates (44; 66.66%) collected from different sources, including standard strain, with 20 samples (44.44%) from food, 8 (17.77%) from food handler, and 15 (33.33 %) from clinical specimens.

The cluster I was divided into two subclusters, IA and IB, with 28% similarity. The subcluster IA contained 32 (71.11%) isolates of *S. aureus* and it was divided into two subclusters, IA1 and IA2. The results revealed that the degree of similarity between the subcluster IA1 whichconsisted of18 isolates (from different food samples) and the 13 isolates (from different samples with *S. aureus* MU50) of subcluster IA2was 32%.

Fig 9. Dendrogram showing the percentage of genetic similarity betweenall isolated from (food, food handler and clinical) samples, SmaI digestion PFGE patterns, with the use of the approach of the unweighted pair group with the arithmetic average values (UPGMA).

The subclusters IA1 was divided into two subclusters, IA1a and IA1b. The degree of similarity between the isolates of subcluster IA1b and subcluster IA1a was 45%. The subcluster IA1a was divided into two subclusters, IA1a1and IA1a2. The percentage of similarity between the isolates of subcluster IA1a1 (12 isolates from different food samples) and the one isolate of subcluster IA1a2 was 60%. The subcluster IA1a1 was divided into two subclusters, IA1a1a and IA1a1b. The percentage of similarity was 62 % between the subcluster IA1a1a which consisted of 4 isolate (S1, S2, S4, S7) and the isolates of subcluster IA2a1b (S5, S6, MP5, S9, MP6, CC4, CA2), which belonged to different food samples.

The subcluster IA2 was divided into two subclusters, IA2aand IA2b. The similarity was 36% between the isolates of subcluster IA2a (10 isolates) and the isolates of subcluster IA2b (3 isolates) which belonged to the food handler samples.

According to the results of the molecular study, the isolates of subcluster IA2a showed a similarity of 38% and were classified into two sub clusters. The subcluster 1A2a1 contained 7 samples while the sub cluster 1A2a2 consisted of 3 isolates, showing a similarity of 47 % from these isolates (67) belonged to standard strain and other two isolates (S10, H6) showed similarity in parentage 82 %. The subcluster IA2b also contained 3 isolates (H3, H7, H8) that belonged to food handler samples, showing a similarity of 52%, while the similarity between the isolates H3 and H7 was 80%.

The subcluster IA2a1 was divided into two subclusters, IA2a1a and IA2a1b. The percentage of similarity was 52 % between the subcluster IA2a1a which consisted of 4 isolate (DP5, CL2, H4, CL1) and the isolates of subcluster IA2a1b (44, 45, 48) which belonged to food handler samples. The subcluster IA2b was divided into two subclusters, IA2b1 and IA2b2. From the results, we observed that the degree of similarity between the isolates of subcluster IA2b1 (H3, H7) and subcluster IA2b2 (H8) was 52%

depending on their classification to the food handler.

The second subcluster IB included 13 (19.69 %) isolates of *S. aureus* which all belonged to clinical samples. It was divided into two subclusters, IB1 and IB2. The percentage of similarity between the subcluster IB2 which contained 7 isolates and the 6 isolates of subcluster IB1 was 80%.

The cluster II included 23 (34.84%) isolates from different food sources. The cluster II was divided into two subclusters, IIA and IIB, with a similarity of 40%. The results showed a similarity of 52% among the isolates of subcluster IIA, which was divided into two subclusters, IIA1 and IIA2. The results revealed that the degree of similarity between the 8 isolates of subcluster IIA1 was 67%. This subcluster was divided into 11A1a which consisted of 2 isolates (CC1, CC3) in percentage 93% and 11A1b which contained 6 isolates (CM2, CE1, CR3, CC2, CK1,CM1) in percentage 82%, with a high similarity between CM2 and CE1 isolates. On the other hand, the subcluster IIA2 contained 6 isolates (CE2, CR2, CR1, CR4, CP1, CR5) with similarity 90% and 100% between the 2 isolates (CE2, CR2). The isolates of the second major subcluster IIB (CC4, CC5, CA3, CA2, MP5, MP4, S9) were similar by 71%, and it was divided into two subclusters, IIB1 and IIB2, with a similarity of 76%.

The subcluster I1B1 was divided into two subclusters, I1B1a and I1B1b. The degree of similarity was 92% between the isolates of subcluster 1IB1b (MP3, DP3, DP1, DP4, CA1, DP2) and subcluster 1IB1a which contain two isolates (MP1, MP2) in percentage 100%.

The MRSA isolated from different food sources, food handler, and clinical samples showed similar patterns due to the common genetic characteristics between these isolates. However, changes in these genetic characteristics may occur easily [13].

PFGE is the most common tool of molecular typing that is utilized in advanced molecular genetics laboratories around the world. It is viewed as the chosen approach for DNA finger printing of MRSA isolated from food products. The typing has a significant part to understand MRSA epidemiology and evaluate the efficiency of the antimicrobial prescribing measures and infection control [23].

Also, one of the programs that are commonly utilized in PFGE is the Pulse Net program of the CDC. The Pulse Net can be defined as a public health labs network that submits the isolates involved in suspected outbreaks of the FBDs for the purposes of molecular typing and comparing to other isolate types in a data base. The PFGE may be utilized with success for the identification of outbreaks, determining the number of the isolates which are involved, and tracking the source these isolates or the outbreak source [24].

The PFGE drawbacks involve the fact that it is relatively expensive, time consuming, and technically demanding. It could be requiring 3-4 days for completing one analysis [11].

Despite these disadvantages, PFGE, when compared with other approaches in a variety of researches, was found to be beneficial in characterizing the isolates of the outbreak [25].

4 Conclusions

Pulsed electrophoresis is considered by many authors the gold standard for the molecular typing of a wide range of microorganisms and for numerous pathogenic bacteria it has proven to be highly discriminatory and comparable, if not superior, to other methods.The results of the this study, although affected by a limited number of food handlers strains, confirm the presence of MRSA and show the great genomic variability even within of methicillin-resistant strains that could spread from handler into the environment involving animals other than humans. This study highlighted the need to undertake measures to monitor diverse MRSA isolates (clinical, food, handler and environment isolates) so that effective intervene effectively to interrupt the epidemiological chain. These findings suggest that the MRSA may be rapidly transmitted from food handlers to food and pose serious public health risks.

5 Recommendations

PFGE is a useful technique for differentiating individual isolates and may be employed as a complement for phenotypic methods and other molecular approaches for epidemiological purposes. It is now the time to evaluate the use and applications of molecular typing methods for typing other foodborne bacteria of public health importance.

6 Acknowledgement

Many thanks to everyone who contributed to the completion of this work, in particular Directorate of the Food Contamination Research Center at the Ministry of Science and Technology

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