Using Random Amplified Polymorphic DNA (RAPD) Fingerprinting Technique to Analyze Genetic Variation in Staphylococcus Aureus Isolated from Different Sources in Babylon Province Hospitals

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ABSTRACT

Genetic fingerprinting of 19 different isolates of *Staphylococcus aureus* from wounds, skin, nails and urinary tract infection taken from patients who admitted at AL-Hilla General Teaching Hospital and Babylon Hospital for Maternity and Pediatric, using random amplified polymorphic DNA (RAPD) was carried out. Two (OPY-07 and OPX-20) primers showed polymorphism among the isolates tested generating 12 bands, 8 of which were polymorphic with sizes ranging between 250 and 1 kb. All the isolates were classified completely into one major group with nineteen different subgroups. The nineteen different subgroups suggest adaptation of *S. aureus* in the different host cells. This indicates possible relationship between host origin and genetic variation among *S. aureus* isolates. The DNA fingerprint defined for each strain of *S. aureus* could be useful in epidemiological studies, medical diagnosis and the identification of new strains and their origins.

Keywords: *Staphylococcus aureus, RAPD, PCR, Fingerprinting Technique, OPY-07 and OPX-20*

Introduction

The major sources of *S. aureus* in hospitals are septic lesions and carriage sites of patients and personnel. Carriage often precedes infection. The principal mode of transmission is via transiently contaminated hands of hospital personnel. Airborne transmission seems important in the acquisition of nasal carriage [1]. *S. aureus* are Gram positive cocci in clusters belonging to Micrococaceae family. *Staphylococcus aureus* is an important agent of healthcare-associated and community-acquired infections; they cause a variety of superficial and deep infections [7]. They are frequently found as contaminants in clinical specimens taken from the body surfaces, for example, swab from skin, nose, throat, wounds, burns and bed-sores. Sometimes, acts as opportunistic pathogens and cause infections. The pathogenicity of *S. aureus* include, abscesses, boils, conjunctivitis especially in newborn, cross-infections in hospitals septicemia and food poisoning [2 &17].

Staphylococcus pathogenic versatility is compounded by its ability to develop resistance to new antibiotics almost as fast as they are introduced and this consider a serious setback in many hospitals causing various hospital outbreaks has been reported in many studies[3]. However, nosocomial infections caused by *S. aureus* are clinically serious and control of such infections requires strain typing to identify degree of virulence, the source of contamination, and resistance to commonly used antibiotics. It is important in epidemiology and ecology to be able to identify bacterial species and strains accurately[18].

Rapid identification and classification of bacteria is normally carried out by morphology, nutritional requirements, antibiotic resistance, isoenzyme comparisons, phage sensitivity[19] and more recently by

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DNA based methods, particularly rRNA sequences or rDNA [20], strain-specific fluorescent oligonucleotides [16] and the polymerase chain reaction (PCR)[15]. Each of these methods has specific applications and advantages. However, closely related isolates are difficult to identify and differentiate using the biochemical methods. For effective chemotherapeutic treatments of infections or disease caused by this organism, the degree of virulence of different strains needed to be determined [2].

The aim of this study is to carry out a genetic characterization of different isolates of *S. aureus* from different sources in Babylon Province hospitals using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). This RAPD procedure works with anonymous genomic markers requires only small amounts of DNA and when compared with the biochemical methods, are simpler, very sensitive, cheaper, faster and less labour intensive than other DNA maker methodologies.

Material and Method

Specimen Collection and the Identification of *S. aureus*: Forty clinical samples were collected from wounds, skin, nails and urinary tract infection taken from patients who admitted to AL-Hilla General Teaching Hospital and Babylon Hospital for Maternity and Pediatric, during the period from October 2017 to the May 2018. Collected samples were serially diluted and spread on sterile Mannitol Salt Agar medium and incubated at 37° C for 24 hrs. Yellow color colonies obtained were screened for the conformation of *S. aureus* by Gram staining, and biochemical (Mannitol fermentation and catalase) tests [14].

Isolates Propagation: About 200 µl *S. aureus* isolate was transferred into 75 ml of nutrient broth (pH 7.5) in a 250 ml conical flask and kept under constant shaking at 37° C for 24 h. The bacterial cell was removed by centrifugation, washed with 0.1 mM Tris-EDTA and kept at -20° C for DNA extraction [13].

Genomic DNA Extraction: DNA of *staphylocus aureus* isolates was extracted and purified according to Geneaid protocol of presto™ Mini gDNA Bacteria Kit,Taiwan. Concentration of DNA was determined spectrophotometrically (NanoDrop) by measuring its optical density at 260 nm the purity of DNA solution is indicated by ratio of OD260/OD280 which is in the range of 1.8 ± 0.2 for pure DNA [11]. Gel electrophoresis was used for detection of DNA by UV transilluminator (Cleaver, UK) [9].

RAPD-PCR amplification: RAPD analysis was according [8], each isolate was tested with Two arbitrary or random primers as described in [Table -1] [18] were primers synthesized by BioNEER, Korea.The DNA amplification reaction was carried out in a 25µl volume containing 5µl DNA, 12.5µl GoTaq® Green Master Mix (Promega), 2.5µl of primer (10 pMol), and 10µl of nuclease free water; A single primer was used in each reaction. Amplification was carried out in thermal cycler (Eppendorf) which was conducted as in [Table-2][18] with a little modification.

Table 1: Oligonucleotide primers that showed genetic discrimination among the *S. aureus* isolates using RAPD-PCR analysis

Primer	Nucleotide sequence	No of fragments amplified	No of polymorphic bands
OPY-07	**5- CTGGACGTCA-3**	5	3
3OPX-20	**5- CCCAGCTAGA-3**	7	5
	Total	12	8

Table 2: PCR program that apply in the thermocyler [18]

Temperature̊ c/time				
Initial denaturation	**Denaturation**	**Annealing**	**Extension**	**Final extension**
94˚C/3minutes	94˚C/1minutes	40˚C/1minutes	72˚C/2minutes	72˚C/7minutes
	Cycles number 45			

The amplification products were resolved by electrophoresis in a 1.2% agarose gel using TBE buffer 1X (at 70 V for 2 h.) A 1 kb ladder (Promega, USA) was included as molecular size marker. Gels were visualized by staining

with ethidium bromide solution (0.5 µg/ml) and banding patterns were photographed over UV light using Gel documentation [9].

Phylogenetic Analysis: Positions of unequivocally scorable RAPD bands were transformed into a binary character matrix (“1” for the presence and “0” for the absence of a band at a particular position). Phylogenetic tree was created by the unweighted pair-group method arithmetic (UPGMA) average cluster analysis [6].

Results and Discussion

Identification of *S. aureus*: A total 40 clinical samples were collected from patients who admitted at AL-Hilla General Teaching and Babylon Hospital for Maternity and Pediatric hospitals. The result has been show 19 isolates as *S. aureus.*

RAPD analysis of *staphylococcus aureus*: Polymorphism assay for *Staphylococcus aureus* isolates was carried out using two primers (OPY-07 and OPX-20); as shown in (figure 1 and 2) respectively.

Figure 1: RAPD-PCR using the primer OPY-07. [M Line (Marker), (1-19) the isolates numbered]

Figure 2: RAPD-PCR using the primer OPX-20[M Line (Marker), the (1-19) isolates numbered]

Genetic characterization of *S. aureus* isolates by RAPD analysis: Two primers (OPY-07, OPX-20) showed polymorphisms among individuals; The amplification reactions with the 2 primers generated 12 bands, 8 of them being polymorphic (Table 1) with ranging between 250bp and 1 Kb detected among the 19 *S. aureus* isolates and each of primer give different genetic profiles (figure 1 and 2).

Using 8 RAPD markers to construct phylogenetic relationship among 19 *S. aureus* isolates with OPY-07 led to classification into one major group while thirteen different subgroups were obtained at 100% similarity coefficient; in these subgroups strain with closed related and divergence as shown (Figure 3).

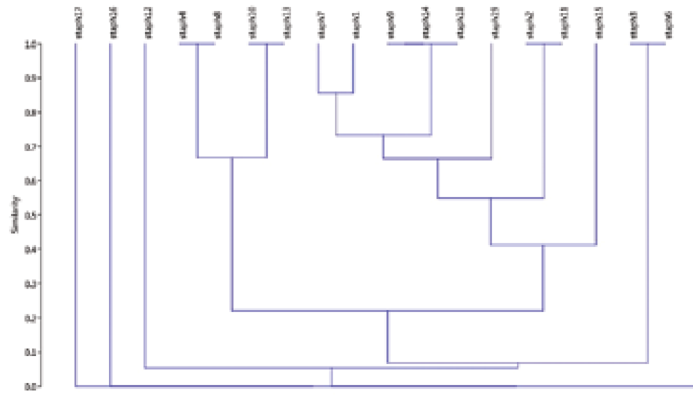

Figure 3: Dendogram analysis showing phylogenetic diversity of 19 Staphylococcus isolates identified by RAPD markers

As with OPX-20 led to classification into one major group while ten different subgroups were obtained at 100% similarity coefficient; in these subgroups strain with closed related and divergence as shown (Figure 4).

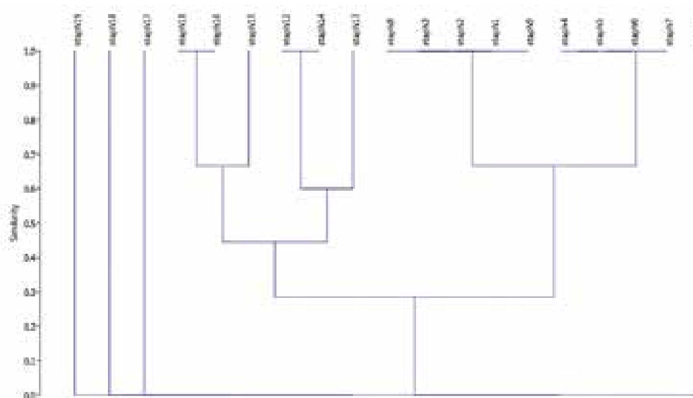

Figure 4: Dendogram analysis showing phylogenetic diversity of 19 Staphylococcus isolates identified by RAPD markers

While phylogenetic relationship among 19 *S. aureus* isolates with (OPY-07, OPX-20) led to classification into one major group while nineteen different subgroups were obtained at 100% similarity coefficient (Figure 5). This level of clustering was based on the origin of *S. aureus* strains.

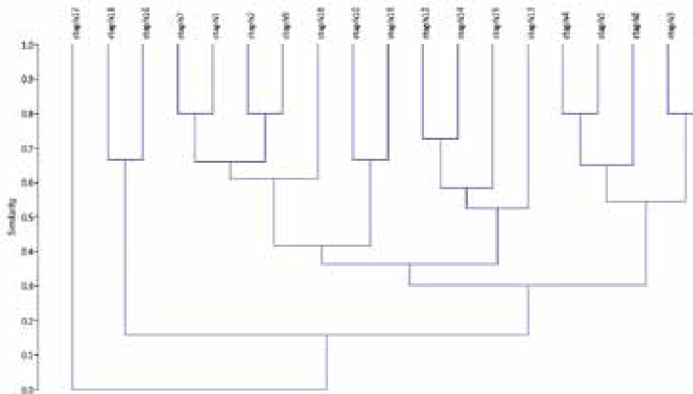

Figure 5: Dendogram analysis showing phylogenetic diversity of 19 Staphylococcus isolates identified by RAPD markers using OPX-20, OPY-07.

Genetic fingerprinting and phylogenetic diversity between different *S. aureus* isolates were determined by converting RAPD data into a Jaccard similarity matrix and analyzed by UPGMA to produce a phylogenetic tree. The DNA band pattern obtained is similar to a bar code, allowing the identification of each individual. For instance, isolate Staph14 and Staph15 presents unique bands when its DNA amplified with OPX-20 primer tested (Figure 2). In the same way isolate Staph1 and 9 with OPY-07 primer (Figure. 1).

These bands could be used to characterize and identify it. All the isolates were classified completely into one major group with nineteen subgroups. However, the nineteen different subgroups obtained in this study suggests possible and frequent occurrence of mutants in *S. aureus* in different host cells. Previously, *S. aureus* has been described as a variable bacterium with many pathogenic and antibiotic resistance variants [17]. The restricted number of cultural and morphological characters of *S. aureus*, and the lack of standardization of cultural conditions and virulence tests among different investigator have led to confusion in the characterization of this pathogen, special phenotypes usually consist of isolates that are genetically less related and such identification of isolates using biochemical, cultural and morphological techniques often lack consistency and accuracy[5]. In the current study, we have found that identification of genetic diversity in *S. aureus* depends on sources of isolates, different host cells and occurrence of mutants and that agree other studies[10].

Beside, the possible and frequent occurrence of mutants in *S. aureus* constitutes the broad genetic variation that exists within Staph1 toStaph19 genotypes (figure 5). RAPD markers exposed possible relationship between host origin, mutation and genetic variation among *S. aureus* isolates, and this proved its fingerprinting and diagnostic potential. Obviously, for these DNA bands patterns to have a practical meaning in the areas of medicine, population biology and epidemiology, specific DNA bands must be related to host origins, mutation and virulence genes[4].

This could be by a systematic comparison of DNA band patterns among bacteria comparing for the different host origins, mutation and virulence genes present. The DNA fingerprint defined for each strain of *S. aureus* should be useful for epidemiological surveys, medical diagnoses, and in the identification of new virulent strains and their origin. Furthermore, RAPD-PCR could be used to track the paths of transmission, which could be used in controlling the spread of strains within hospital, and between the hospitals, and especially preventing the nosocomial infections caused by the multi-drug resistant MRSA[12].

Conclusion

Random amplification of the DNA of *S. aureus* isolates reveals the efficacy of these selected nucleotides sequences in determination the similarity or variations among all isolates.

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Conflict of Interest: There are no conflicts of interest

Ethical Clearance: Permission to conduct this study was issued by the Health institutional; AL-Hilla General Teaching Hospital and Babylon Hospital for Maternity and Pediatric, and the Swabbing from patients was carried out by a public health technician.

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